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PARTIAL STERILISATION OF SOIL
BY ANTISEPTICS.

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INTRODUCTION.

THE work described in this paper has been carried out at Rothamsted during the years 1918–21, and was undertaken in direct continuation of earlier work on Partial Sterilisation of Soil, begun by Russell and Hutchinson (2, 3) and carried on by Buddin (6).

Comparison of the influence of various chemicals on soil organisms has led to conclusions which differ from those advanced by the authors mentioned above and are somewhat simpler.

Russell and Hutchinson (2, 3), after experiments on field soils of varying richness with a water content of 15–19 per cent., supplemented to some extent by experiments on rich greenhouse soils, advanced a most interesting theory. This Partial Sterilisation theory is briefly as follows. The bacteria in the soil are kept in check by the protozoa which share their environment. Could the protozoa by some suitable means be eliminated, then the bacterial numbers would inevitably rise, and in consequence the fertility of the soil would be improved.

They demonstrated rises of the number of bacteria after treating soil with toluene, carbon bisulphide and steam, and showed that the fertility of the treated soil was much increased while the ammonia and nitrate content was heightened, these effects being always obtained at a dose which also caused the death of the larger protozoa.

The greater part of the work now described was done on rich soil from cucumber and tomato houses, the moisture of which is very high— varying from 32 to 42 per cent. There is also a very high nitrate content, 300 to 700 parts per million of nitric nitrogen against 10 to 40 in field soils. The addition of various chemicals to these soils causes high rises in bacterial numbers, whether the whole of the dose is left in the bottle, or whether (in the case of a volatile chemical) it is later allowed to escape

by spreading the soil out in a thin layer (Russell and Hutchinson Method). Similar rises follow in field soil, but the time taken to produce a given effect is far longer.

The results obtained for greenhouse soils indicate clearly that such rises are for a limited period only, and of the nature of a temporary disturbance of equilibrium among the bacterial population. The bacterial numbers rise to a maximum and then slowly fall again, and there is good reason to suppose that they return to the original control numbers. The decline is often very long drawn out with the higher doses; *e.g.* using cucumber soil, the $M/10$ and $M/50$ doses of benzene and toluene cause bacterial rises which remain high above the normal level even after 190–250 days, although gradually and continuously falling (Figs. 1 and 7).

As mentioned, the time occupied by similar rises and falls in field soil is much longer, and so when Russell and Hutchinson found rises lasting for many months they considered them permanent, and owing to the removal of detrimental protozoa.

Examination of the graphs of bacterial numbers accompanying this paper will show that a large part, if not all, of the increase is only transient, and that the fall is due to the exhaustion of the chemical on which the bacteria have been feeding and possibly of the products of their activity in the soil[1].

METHODS EMPLOYED.

The method employed by Russell and Hutchinson and later by Buddin was as follows(2, 3):

(1) The soil was mixed with the desired dose and kept in a corked bottle for a definite time—usually 48 hours.

(2) Then it was spread out on sterile paper and lightly covered with another sheet so that the antiseptic if volatile could escape. (This would permit infection and complications due to alteration in moisture content, etc.).

(3) After an arbitrary interval, usually 24 hours, ending when such a chemical as toluene ceased to smell, the soil was returned to the bottle, re-moistened, and left for many days with a cotton-wool stopper replacing the cork to ensure free interchange of air.

(4) Bacterial counts were then made, at somewhat long intervals

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[1] When the bacterial numbers obtained by Russell and Hutchinson by adding toluene to bottles of soil, and making counts at intervals for 500 days are plotted against the untreated soil numbers, it is quite obvious that the curve agrees with the curves accompanying this paper. Due regard must of course be paid to the slow rate of change in field soils. The curve is largely a record of the *slow utilisation* of toluene.

for it was believed that the whole of the antiseptic had evaporated. Absence of smell, however, is no proof that there was not left in the soil after airing a certain amount of adsorbed toluene as well as oxidation products of toluene, since spreading out in a thin layer would facilitate any oxidation that was progressing. It is to the slow utilisation of these products in soil that the bacterial rises obtained when antiseptics are added must now in very great part be attributed. There may also be some rise comparable with that caused by steam, lime (4) or dry heat where stores of food not hitherto available are rendered soluble, and again some slight further rise may occur owing to bacteria feeding on dead protozoa.

The utilisation of such compounds as vanillin (11), cumarin, alcohol, pyridin, cresol (6) and sugar (13) by bacteria is by no means a new idea, though it may at first seem rather surprising for such a series as the aromatic hydrocarbons. Yet a careful study of the graphs accompanying this paper leaves no alternative way of considering the matter.

The earlier work done for the present paper was carried out by the Russell-Hutchinson method, but presently it was realised that experimental data so gathered allowed of no comparison between volatile and non-volatile antiseptics. Bacterial numbers obtained in the latter case were attained in the presence of a known administered dose of chemical, but in the former were yielded by an *indefinite* amount of reagent and may be due partly to a residue left in the soil and partly to the effect of the whole dose while present. With the less volatile substances airing will produce an effect intermediate between such extreme cases as benzene and lime, and it will alter with the volatility of the reagent. Therefore a fresh series of experiments was made wherein the antiseptics were left in the soil, and comparative work thus being rendered possible new light was rapidly thrown on the whole question. Comparison was also facilitated by adopting Buddin's method (6) of adding the compounds in definite fractions of a molecular weight per kilogram of dry soil.

$$\frac{\text{Molecular weight}}{10}, \quad \frac{\text{Molecular weight}}{50} \quad \text{and} \quad \frac{\text{Molecular weight}}{100} \quad \text{per kilo,}$$

or shortly $M/10$, $M/50$ and $M/100$ were the doses usually employed.

Unfortunately the bacterial estimations made by Russell and Hutchinson and Buddin were not frequent enough, as subsequent investigation proved, to represent the whole course of events after adding the reagent. Many high rises and steep falls are very quickly over, and may be missed entirely during an interval of ten days or less between two successive counts. Thus the bacterial graph will be faulty and inadequate, for any

two points on the curve may give no hint as to the true course of the line joining them.

As soon as an attempt was made to compare the graphs of bacterial rises caused by adding the aromatic hydrocarbons, it was plain that the same soil should be used throughout all such experiments, and that counts should be made at equal intervals of time. As the numbers in the control soils themselves varied between 30 and 150 million bacteria per gram of dry soil, it was only possible to compare graphs of different compounds by plotting the *difference* between the numbers in treated and untreated soil, instead of the actual numbers (Figs. 18 and 19).

The work of Hiltner and Störmer [1] and of Greig Smith [8, 9] was found very helpful in explaining the results now presented. Study of the gelatin plates obtained with different reagents and at different doses of the same reagent strongly supports the views of Hiltner and Störmer. They believe that on adding carbon bisulphide to soil unequal retardation is caused in the growth of the different groups of bacteria, some groups becoming disproportionately prominent, and others almost entirely suppressed. Speaking generally of the various substances treated in this paper, the disturbance which leads to screening or even suppression of some bacteria and abnormal preponderance of others sooner or later wanes, and ends. Specific bacteria.do seem to attack specific substances or groups of substances, the number of species decreasing as the dose rises, whereas within certain limits the numbers attained by any one species go up. When the reagent has been destroyed, and the numbers return to normal, in many cases all the normal soil species are still present (*e.g.* in the case of benzene and naphthalene), so that few or no types have actually died. In other cases the general type has changed, and some bacteria have permanently disappeared as though killed by the chemical (*e.g.* chlordinitrobenzene).

There is not a sharp "lock and key" effect of one specific bacterium which is alone able to attack one specific substance; the specialisation is far less narrow. Very similar bacteria appear to flourish on (or withstand) pinene, naphthalene and pseudocumene for instance—*Bacillus liquefaciens* being very prominent among these, as there seem very few substances that this resistant bacterium will not attack or tolerate.

Hiltner and Störmer also suggested that the rapid increase in bacterial numbers would be followed by a more intense transformation of plant food substances, and that by decomposition and fixation processes, an accumulation of readily available nitrogen compounds would be brought about, which could be utilised by the crops. Their work furnishes a clue

to the real cause of the bacterial rises hitherto attributed to Partial Sterilisation.

Greig Smith[8] found that when the Russell-Hutchinson method was used all the antiseptic applied was not removed in such cases as that of chloroform. He considers that traces remain which can themselves stimulate bacterial development. In a further paper dealing with the action of toluene[9] he states that the water content of the soil modifies its effect on the protozoa. Even 20 per cent. toluene is unable to kill them if the soil is less than one-tenth saturated with water, whereas 1–2 per cent. will kill ciliates but act irregularly on amoebae and flagellates if the soil is more than one-tenth saturated. These facts are probably linked up with the presence of the protozoa as spores or otherwise when the toluene is added. With his opinion as to the irregular action of toluene (and other antiseptics) on amoebae the writer heartily concurs. For rich cucumber soil higher doses of a reagent are always necessary to kill protozoa, than when the poorer field soils are used, as though the high organic content acts as a buffer and hinders the effectiveness of the chemical[1].

It is to be clearly understood that the present paper does not in any way dispute the *facts* presented by Russell and Hutchinson but views them from a different standpoint. Their improvements in crop results may be caused by ammonia and nitrate elaborated by the particular groups of bacteria which any specific reagent favours, and not only by the elimination of any *detrimental factor*. Or again, improvement of crop may result from more rapid maturing of the soil[10] since the wave of disturbance in the bacterial content may cause specially rapid breaking down of plant residues, etc.

Hearty thanks are due to Mr Tattersfield, Organic Chemist at Rothamsted, for his constant and kindly encouragement during the progress of this work.

[1] The accompanying table shows that higher doses of reagents are necessary to kill amoebae in cucumber soil than in field soil. The doses in field soil are quoted from Buddin's paper.

Chemical		Toxic dose to amoebae	
		Field soil (Buddin)	Cucumber soil
O. cresol	... ...	$M/50$	$M/2$
Toluene	... ...	$M/200$	$M/10$
Benzene	... ...	$M/50$	$M/10$
Formaldehyde	...	$M/100$	$M/50$

<center>PART I.</center>

Description of the effect on soil organisms when various reagents are added to soil, together with changes in the ammonia and nitrate content.

Table I is a full list of the reagents tested by the writer, but complete description of all these would make the paper unwieldy and the cost of production prohibitive and therefore detailed discussion has been confined to the following substances:

Benzene	Toluene	Carbon disulphide
Naphthalene	Xylene	Phenol
Hexane	Formaldehyde	Cresol

Benzene and naphthalene will first be discussed as typical examples. They were examined for their effect on field and cucumber soil kept in corked bottles, and benzene also for its effect on cucumber soil when the experiment was carried out in the Russell-Hutchinson way. A very marked difference was found according to the method used. In the latter case benzene did not give any very high bacterial numbers, 408 million being the highest count obtained during the 62 days of the experiment, while a level of 3080 millions was reached by the 71st day when the same dose of benzene was allowed to remain in the soil.
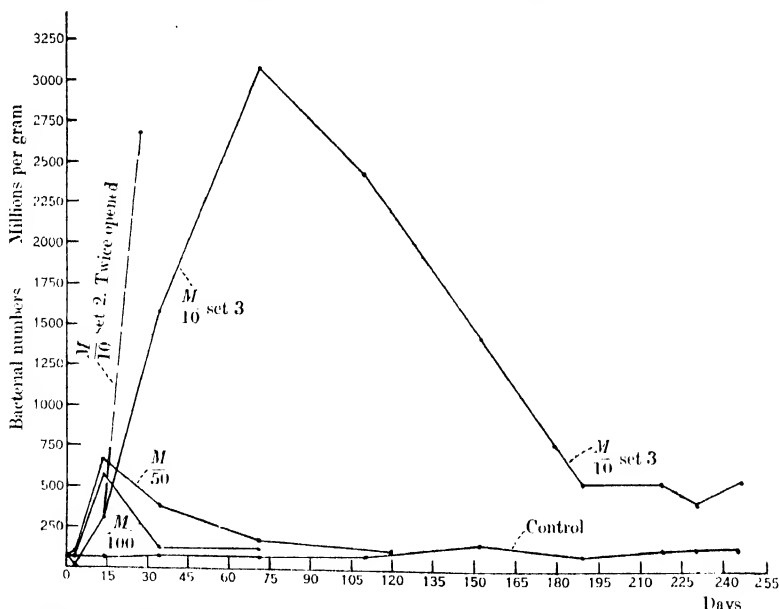
Fig. 1. Cucumber soil treated with benzene, not aired off; 48 % moisture.
Bacterial numbers per gram of dry soil.

Table I. *Reagents tested.*

Aliphatic compounds

Name	Formula
Formaldehyde * M	H.CHO
Acetaldehyde *	CH₃.CHO
Hexane *	C₆H₁₄
Cyclohexane	C₆H₁₂
Pentane	C₅H₁₂
Chloroform	CHCl₃
Chlorpicrin * M	C.Cl₃.NO₂
Ethyl alcohol	C₂H₅OH
Acetone	CH₃.CO.CH₃
Methyl ether	CH₃.O.CH₃
Ammonia R	NH₄OH
Sodium cyanide R	NaCN
Carbon disulphide	CS₂

Aromatic compounds

Name	Formula	Diff. from parent substance
Benzene * M	C_6H_6	—
Toluene * M	$C_6H_5.CH_3$	CH_3
Xylene * M	$C_6H_4(CH_3)_2$	$(CH_3)_2$
Pseudocumene M {	$C_6H_3(CH_3)_3$	$(CH_3)_3$
Mesitylene M {		
Cymene * M	$C_{10}H_{14}$	$CH_3 . CH(CH_3)_2$
Pinene * M	$C_{10}H_{16}$	
Naphthalene *	$C_{10}H_8$	
Thymol	$C_{10}H_{14}O$	
Chlorbenzene	$C_6H_4.Cl$	Cl
Dichlorbenzene, o- and p-	$C_6H_4.Cl_2$	2Cl
Nitrobenzene R	$C_6H_5.NO_2$	NO_2
m-Dinitrobenzene	$C_6H_4(NO_2)_2$	$2(NO_2)$
o-Chlornitrobenzene	$C_6H_4ClNO_2$	$Cl.NO_2$
Chlordinitrobenzene	$C_6H_3Cl(NO_2)_2$	$Cl(NO_2)_2$
Nitrodichlorbenzene	$C_6H_3.NO_2Cl_2$	NO_2Cl_2
Dichlordinitrobenzene	$C_6H_2(NO_2)_2Cl_2$	$(NO_2)_2Cl_2$
Phenol	$C_6H_5.OH$	OH
Chlorphenol R	$C_6H_4.Cl.OH$	Cl.OH
Picric acid R	$C_6H_2.OH.(NO_2)_3$	$OH.(NO_2)_3$
Cresol R	$C_6H_4.CH_3.OH$	$CH_3.OH$
Chlorcresol R	$C_6H_3.Cl.CH_3.OH$	$Cl.CH_3.OH$
Dichlorcresol R	$C_6H_2Cl_2.CH_3.OH$	Cl_2CH_3OH
Anilin R	$C_6H_5.NH_2$	NH_2
p-Nitranilin	$C_6H_4.NO_2.NH_2$	$NO_2.NH_2$

Mixtures

Mixtures	
Naphtha from gas works	R
French cymène. coal tar product	R
Calcium sulphide and naphthalene (equal parts)	

Inorganic substances

Inorganic substances	
Lime	R
Calcium sulphide	
Steam *	

* Indicates that the substance has been tested on both field and greenhouse soils.

M Indicates that experiments have been made by the Russell-Hutchinson method and also in corked bottles

R Indicates that experiments were carried out by the Russell-Hutchinson method alone.

BENZENE. Formula C_6H_6. Molecular weight 78. Heat of combustion 800. Density ·879. Boiling point 80·36° C. Vapour pressure 74·7 mm. at 20° C.
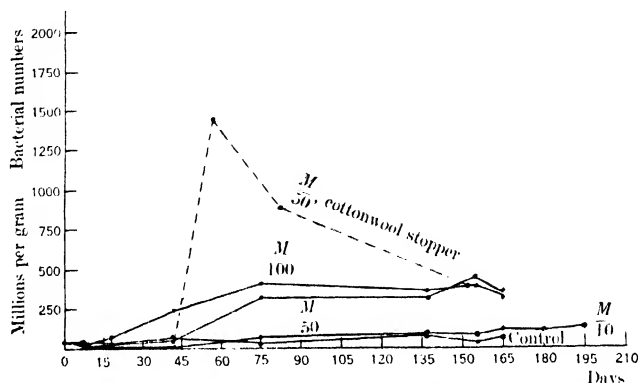
Fig. 2. Field soil treated with benzene, not aired off; 14·6 % moisture.
Bacterial numbers per gram of dry soil.

Exp. 1. Tomato house soil of 26·7 per cent. moisture was used. The $M/100$, $M/50$ and $M/10$ doses of benzene were left in the soil for three days, the cork being by no means air-tight. Then bacterial counts were made, demonstrating the effect of the full dose added less any that escaped owing to volatility. Then the soil was spread out overnight in a thin layer on paper, returned to the bottle, and made up to the original moisture content to correct loss by evaporation; cotton-wool stoppers were now fitted instead of corks.

Table II. *Benzene. Tomato house soil. Russell-Hutchinson method.*

Grams of chemical added per kilo of dry soil	Millions of bacteria present per gram dry soil after			Other organisms present after			Protozoa present
	3 days	37 days	62 days	3 days	37 days	62 days	
Untreated 0	58·5	95·6	76·5	Fungi, eelworm	Fungi, eelworm	Fungi, eelworm	F.C.Am.*
$M/100$ 0·78	55·7	90·3	94·7	do.	do.	do.	do.
$M/50$ 1·56	71·7	90·7	100·8	Fungi, few eelworm	Fungi	Fungi, eelworm	do.
$M/10$ 7·8	15·3	408·0	379·0	Fungi cut down	Fungi cut down	Fungi cut down	Many F., some small amoebae

Soil	Parts per million of nitrate present after			Parts per million of ammonia present after			Parts per million of nitrate and ammonia present after		
	3 days	37 days	62 days	3 days	37 days	62 days	3 days	37 days	62 days
Untreated	305†	349	328	2·8‡	2·0	1·8	307·8	351·0	329·8
$M/100$	321	375	352	7·2	0·7	2·4	328·2	375·7	354·4
$M/50$	281	364	324	19·3	1·2	1·8	300·3	365·2	325·8
$M/10$	286	309	290	18·6	110·7	117·0	304·6	419·7	407·0

* Am. = Amoebae. F. = Flagellates. C. = Ciliates as observed in hay infusion, at each bacterial count.   †‡ See footnotes 1 and 2 on opposite page.

Reference to Table II shows that little rise in numbers occurs at the $M/50$ and $M/100$ doses, but a most marked effect at the $M/10$ dose. This causes first a sharp drop in numbers, followed by a fairly high and sustained rise, and thus the graph of the effect caused by $M/10$ is what Russell and Hutchinson considered typical of the course of true Partial Sterilisation.

Another way of interpreting the results would seem to be as follows. Benzene being very volatile can under the Russell-Hutchinson conditions cause no rise with small doses like $M/50$ and $M/100$. But enough remains in the soil from the $M/10$ dose to produce a sustained rise, the dose being drastic enough to cause an initial depression. During the 24 hours the soil is spread out, and later when cotton-wool stoppers allow free access of air, the benzene remaining in the soil is oxidised and provides energy which results in a heightened bacterial population. The rise in numbers is accompanied by a high rise in ammonia—to 120 parts per million.

At $M/10$ the nitrate[1] + ammonia[2] content of the soil rises about 70 parts per million by the 37th day, the rise being sustained until the 62nd day at least, so that undoubtedly the effect of treatment with the $M/10$ dose of benzene is to increase the amount of readily available nitrogen, during the period the experiment covers.

Protozoa as determined by the 1 per cent. hay infusion method (7a) are modified by the $M/10$ dose, flagellates and some amoebae alone surviving.

Exp. 2. Very different results are obtained if the doses are left in contact with the soil. In the case of cucumber soil (Fig. 1) the numbers rise at the $M/100$ dose from 60 to 560 million in 14 days, and to 660 million at $M/50$ dose. The rise is purely temporary and no doubt associated with the disappearance of the benzene of which there is no longer any smell, and by the 34th day the numbers have dropped again. This drop continues at a much slower rate until the 120th day, when the types and numbers of bacteria on the gelatin plates are once again very like those of the untreated soil, indicating that the wave of disturbance among the soil population is at an end and equilibrium re-established. $M/10$

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[1] Mr D. J. Matthews, formerly of Rothamsted, also determined nitric nitrogen in parts per million, by a new method worked out on material provided by these experiments. The paper (17) is at present in course of preparation and will be published shortly.

[2] Ammoniacal nitrogen was determined by Mr D. J. Matthews by the aeration method he described in a recent number of the *Journal of Agricultural Science* (16). The method was worked out during these investigations, so that figures are wanting for experiments with many reagents. Nitric and ammoniacal nitrogen data were only determined in the case of experiments by the Russell-Hutchinson method. Much useful information as to the ammonia and nitrate variations when the full dose of a volatile substance is left in the soil is therefore lacking.

in an ordinary corked quart bottle causes an initial depression in numbers which by the 14th day gives way to a rise of 300 million. Further steady rises in the bacterial population bring it to 3100 million on the 71st day while all smell of benzene is gone by the 34th day. From this high level the numbers slowly fall, much more slowly than they rose, as is found to be the general rule. Such a slowing down is no doubt due to the greatly increased amount of broken down plant remains, resulting from the activity of the abnormally heightened bacterial population. The numbers in this soil remain at 410 million above control even on the 246th day, though undoubtedly declining, as the graph shows. A striking proportion of these consists of the brown *Streptococcus*, the same fact holding for $M/10$ toluene when the numbers are again nearing control.

Hence as a direct or indirect result of supplying potential energy in the form of benzene the population is far above normal at the $M/10$ dose after 250 days.

Exp. 2 *a.* When field soil is used (Fig. 2), all doses at first depress the bacteria, a rise following on the 18th day at the $M/100$ dose, and not till after the 41st day with the $M/50$ dose. Thus the rises are very slow compared with those in the lighter and better aerated cucumber soil[1], but the numbers remain high much longer, standing at 340 and 400 millions respectively above control from about the 75th to the 165th days, instead of being back at the level of untreated soil as is the case with cucumber soil. Again, the smell of benzene persists for at least 41 days with the two weaker and 165 days at the $M/10$ dose, instead of rapidly disappearing as with cucumber soil.

$M/10$ keeps the numbers below control for 57 days, when a very slow rise begins which even on the 195th day has only reached about 70 million above control.

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[1] Greenhouse soils contain much more moisture, potash, nitrogen and phosphate than do field soils. To test whether an increase of these constituents would lead to quicker utilisation of the benzene and toluene in field soils and so make the rate of bacterial multiplication more like that of a cucumber soil, potassium nitrate and superphosphate were added along with 6 per cent. additional moisture.

Four bottles were put up:

    (a) 6 per cent. moisture added.
    (b) 6   ,,   ,,   +inorganic salts.
    (c) 6   ,,   ,,   +   ,,   +$M/100$ benzene
    (d) 6   ,,   ,,   +   ,,   +$M/100$ toluene

The numbers in (a) and (b) remained alike during the 45 days of the experiment. The rate of rise induced by benzene was much more like that of cucumber soil, but the same did not hold for toluene. Yet as it could be altered for benzene it may be that the inorganic salts present control the rate of multiplication to some extent, although the total surface exposed to air is probably the prime factor.

This detailed description emphasises how slowly things move in field soil, and makes it easy to explain a rise of 40 million above control on the 500th day as described by Russell and Hutchinson for toluene. Enduring so long, it might easily be mistaken for a permanent heightening of the bacterial population, and only the full series of experiments now presented makes it obvious that their bacterial counts were undoubtedly made on the declining side of a wave of bacterial disturbance. This was occasioned largely by energy supplied as toluene left in the soil even after airing overnight.

Naturally the dose has to be large enough to leave sufficient residue of toluene after airing, or big rises in numbers cannot follow. These doses Russell and Hutchinson picked out as "Partial Sterilisation doses," but in addition to any effect due to the killing of protozoa a feeding effect on the bacteria has probably been a large factor in the experiment.

Turning now to several interesting special cases:

1. Effect of hermetically sealing up $M/10$ benzene with 100 grams of cucumber soil, in a flask of about 1·5 litres capacity. Here two modifications of ordinary practice are introduced:

   (a) the benzene is prevented from volatilising away;
   (b) a very limited air supply is permitted, so that oxidation is seriously hampered.

As a result the bacterial numbers in the treated soil remain below the untreated level for more than 42 days. Opening a sealed flask on the 17th day[1] results in a quick rise in numbers which takes place even more rapidly than in ordinary corked bottles.

2. Effect of opening a corked bottle twice (Fig. 1). Four sets of bottles are usually made, so that the bacterial changes caused by the $M/10$, $M/50$ and $M/100$ doses can be estimated four separate times, on hitherto unexamined soil. But a fresh atmosphere enters each time the cork is removed (and a little benzene is lost). This new supply of oxygen makes a serious difference as it accelerates oxidation, and results in a still quicker rise in numbers. Fig. 1 illustrates this; the numbers in $M/10$, set 3, have only reached 1580 million on the 34th day, but in $M/10$ set 2, twice opened, they stand at 2680 million by the 26th day, since quicker oxidation favours quicker multiplication.

These two special cases emphasise the fact that the whole question

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[1] When the sealed flask was opened on the 17th day there was still a very strong benzene smell, but underlying it was a distinct smell like butyric acid. The same smell turned up with the $M/10$ dose of toluene in corked bottles, on the 34th day, underlying the smell of toluene.

of the rise in bacterial numbers, when the soil population is disturbed by the introduction of a new source of energy, is knitted up with the oxygen supply and can be controlled through this factor. Many determinations have necessarily been made from the fourth set of bottles in these experiments, the number of sets being far too small for all the counts made. In such cases the bacterial determinations must be accepted with caution. They can only illustrate the numbers given by the ultimate oxidation of the reagent supplied, and they make the energy appear available much quicker than it really is. In other words, the rate of bacterial change is unnaturally accelerated by repeatedly opening the same bottle instead of using an unopened bottle every time. Still the estimations so made do indicate the final fall in numbers, and make it possible to get fairly complete results in a reasonable period of time.

3. A new series of rubber stoppered bottles was put up, and the cucumber soil treated with $M/10$ benzene in each case. Lead paper covers were added to protect the stoppers from the benzene, and consequently the bacterial numbers in the various bottles jumped up and down in a see-saw manner when the contents were estimated from time to time and a curve plotted. This was undoubtedly owing to irregularity in the air supply caused by bad fitting of the lead foil, and possibly also to the escape of benzene owing to volatility.

4. The $M/50$ bottle in series 3 of field soils treated with benzene, had its cork replaced for six days by cotton-wool on the 50th day of the experiments. This arrangement, ensuring free oxidation, resulted in an enormous rise in bacteria—from 50 million to 1420 million in the following six days (Fig. 2). This is just another case of controlling the rate of oxidation and availability of the benzene by regulating the air supply. Similar treatment of the $M/10$ bottle, series 3, had much less striking results, as this dose is so drastic in action and but slowly utilised in field soil. During the next 110 days a rise to 160 million was the highest recorded, the cork stopper again replacing the cotton-wool after six days as in the $M/50$ bottle. The slowness of the utilisation of benzene in field soil is undoubtedly due to relatively bad aeration. The rate of change is quick in the very light and well aired cucumber house soils.

Action of benzene on protozoa. Determinations were made, using $M/10$, $M/50$ and $M/100$ benzene on a tomato house soil of 26·7 per cent. moisture, and on cucumber soil of 48 per cent. moisture. When the experiment was made on the former in the Russell-Hutchinson way hay infusion cultures showed no effect on protozoa except at the $M/10$ dose (Table II). Here all ciliates were killed, and all but a few very small

amoebae disappeared. But when the benzene was left in contact with cucumber soil for a long time, a different effect was found. Two separate tests were made, one in August, and another in December. In August the $M/10$ dose had been in the soil for 26 days, and in December for 20 days. The mixing may have differed in thoroughness in the two experiments, but certainly the December bottle was well shaken at frequent intervals. Fig. 1 shows that 20–26 days after adding $M/10$ benzene the bacterial numbers stand at 700–1100 millions per gram. Many flagellates, 12 trophic ciliates and 750 trophic amoebae were found per gram in August, while flagellates alone were found in December, so that as Greig Smith states about toluene, the action on amoebae is irregular. However all three experiments agree in proving that $M/10$ is the first dose to cut down protozoa drastically, but apparently the action is influenced by the length of time the reagent is in, and the thoroughness of the mixing. In the December experiment the trophic ciliates were cut down to half by the $M/100$ dose and to a quarter by the $M/50$ dose, while the latter also cut down the trophic amoebae.

To some extent then the enormous rises in bacterial numbers in the case of benzene, go hand in hand with a progressive cutting down of the amoebae and ciliates, yet the mass of evidence in this paper suggests that the effect on protozoa is very probably not the only factor but rather an expression of the increased toxicity of rising doses.

This increasing toxicity is shown also in the action of benzene on fungi and free-living eelworm[1] when the experiment is carried out in the less drastic Russell-Hutchinson method.

Table II shows that the eelworm are cut down by the $M/50$ dose but eliminated by $M/10$. Fungi, always much more resistant, are merely cut down to some extent by $M/10$.

NAPHTHALENE. Formula $C_{10}H_8$. Molecular weight 128. Heat of combustion 1235. Vapour pressure 0·195 mm. at 35°C., 0·06 mm. at 20°C.

This aromatic hydrocarbon, consisting of a double benzene ring, will be described next, because it presents many interesting features which throw light on the utilisation of energy when provided in the form of hydrocarbons or their derivatives.

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[1] The tests for fungi and eelworm were made on potato agar and glucose agar plates. Nine days were allowed at 34°C. for the appearance of these organisms, and non-appearance in this time taken to signify death or at least inhibition. The $M/10$ benzene visibly lessens the fungus growth on a nutrient agar plate, compared with the untreated soil, and eliminates all eelworm. These tests are really qualitative, and only roughly quantitative, a measured spoonful of soil being taken each time. Still the differences between the effects of different doses are so striking as to be worth recording.

Table III. *Exp. 2. Benzene. Cucumber soil of* 48 % *moisture.*

Grams of chemicals added per kilo of dry soil	Million bacteria present per gram of dry soil after						
	3 days	14 days	26 days	34 days	71 days	110 days	120 days
Untreated	66·4	56·1	115·8	79·4	72·9	72·6	112·7
M/100, 0·78 grams	63·4	572·2	—	120·6	122·8	—	—
M/50, 1·56 „	87·2	660·6	—	393·4	178·5	—	122·8
M/10, 7·8 „	17·9	316·8	2675·0	1580·0	3077·0	2423·0	—

	152 days	189 days	214 days	218 days	230 days	246 days	
Untreated	165·4	97	141·0	141	141	141·0	—
M/100, 0·78 grams	—	—	—	—	—	—	—
M/50, 1·56 .,	—	—	—	—	—	—	—
M/10, 7·8 „	1425·0	543	701·5	543	426	555·5	—

Table IV. *Exp. 2 a. Field soil.*

Million bacteria present per gram of dry soil after

Chemical	7 days	18 days	41 days	75 days	137 days	155 days	165 days	195 days
Untreated	45·3	40·6	65·9	37·0	75·0	45·6	56·6	—
M/100	32·6	58·2	237·8	401·0	351·7	389·1	312·3	—
M/50	14·4	39·8	57·4	318·5	312·3	447·7	325·9	—
M/10	7·8	8·2	7·9	62·8	89·5	78·8	107·7	128·8

Table V. *Rubber stoppers.*

Million bacteria present per gram of dry soil after

	5 days	15 days	23 days	40 days	59 days
Untreated	95·3	95·3	95·3	77·4	68·6
M/10	26·2	477·1	133·4	966·5	1675·0

Table VI. *Action of benzene on cucumber soil in sealed flasks.*

Million of bacteria per gram of dry soil after

Dose	17 days	42 days
Untreated ...	177·8	82·2
M/10 ...	54·9	44·0

Table VII. *Field soil. Set* 3. *Cotton-wool stopper replacing cork from 50th to 57th day only.*

Million of bacteria present per gram of dry soil after

Dose	57 days	83 days	151 days	167 days
Untreated	37·5	52·7	52·7	52·7
M/50	1422·0	872·6	391·4	—
M/10	47·2	158·1	128·0	114·0

At the end of this paper a series of tables is added, which analyses the toxic effect of many benzene derivatives on fungi, eelworm, bacteria and protozoa, as shown by the experiments described in this paper.

A preliminary experiment was made on cucumber soil of 47·8 per cent. moisture. The soil was not spread out but after two or three days cotton wool stoppers were substituted for corks. One series of bottles was kept at ordinary room temperature, while another was incubated at 34° C., to see whether the increased volatility of naphthalene at higher temperatures would influence the results. $M/50$ gave the greatest immediate rise at both temperatures, but in all the treated bottles the
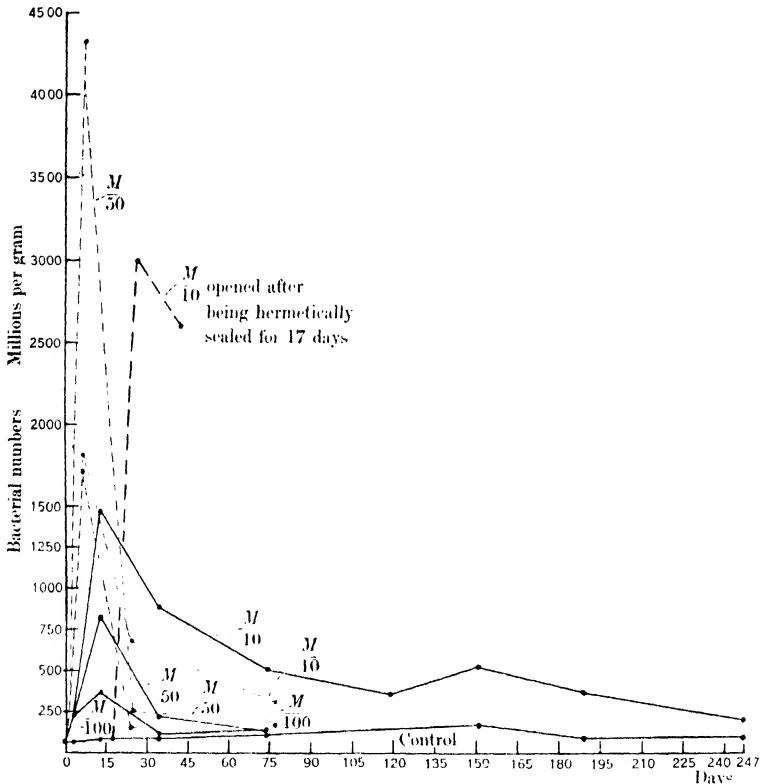
Fig. 3. Cucumber soil treated with naphthalene at 18° C.; 48 % moisture. Full lines: in corked bottles. Broken lines: in bottles with cotton-wool stoppers. Bacterial numbers per gram of dry soil.

numbers increased enormously during the first few days (Fig. 3). At $M/50$ they rose in seven days to 4500 million at 18° C. and 1200 million at 34° C., while $M/100$ and $M/10$ gave 1817 million and 1912 million per gram dry at 18° C., but only 520 and 550 million respectively at 34° C. Therefore raising the temperature to 34° C. checks the rate of bacterial multiplication very severely, although the volatility is increased; but

then even in untreated soil[1] the numbers drop from 127·2 million to 33 million per gram of dry soil when incubated for 74 days at 34° C., this drop being accompanied by a rise in the nitrate from 576 to 1003 parts per million since 34° C. is near the optimum for nitrification.

At 18° C. the high bacterial numbers rapidly fell again after the seventh day and by the 24th day in $M/100$ they were only 60 million above control while $M/50$ stood at 135 and $M/10$ 700 million above control. The fall continued at a much slower rate until the 77th day when the experiment was discontinued.

A similar course was followed in the series of bottles incubated at 34° C., but the return to the untreated level was accelerated, and the numbers were

Fig. 4. Cucumber soil treated with naphthalene; 47·8 % moisture. Russell-Hutchinson method. Difference of nitric nitrogen from control in dry soil.

all very near normal on the 74th day. No ammonia is produced at 18° C., but at 34° C. the ammonia in the $M/10$ and $M/50$ bottles rises from two parts to 67 and 32 parts by the sixth day, and has disappeared
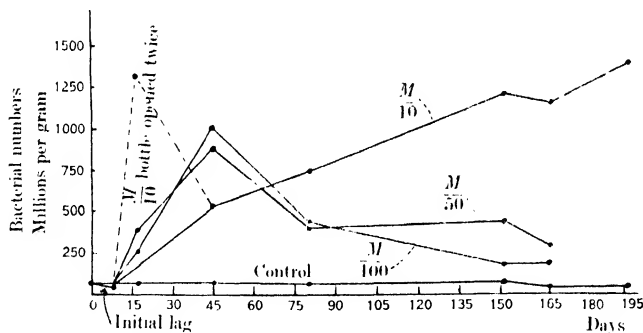
Fig. 5. Field soil treated with naphthalene; 14·6 % moisture. Bacterial numbers per gram of dry soil.

again by the 28th day (Table VIII). This rather indicates that at 34° C. the naphthalene temporarily paralyses the nitrifying bacteria, but that after it has been in large part destroyed they revive, and at 34° C. which

[1] These results are quite in accordance with those described by Russell and Hutchinson[3], who examined the effect of incubation at higher temperatures than 18° C., on partially sterilised soils.

is near their optimum temperature, soon nitrify the accumulated am-
monia. As with phenol and xylene, the rises in numbers are clearly
reflected in a drop in the nitrate content, this fall increasing with the
dose and disappearing when the numbers approach normal again (Fig. 4).
Curiously enough, comparatively little call is made on the nitrate at
$M/100$, although the bacterial numbers reach 1817 million on the seventh
day. The nitrogen of the nitrate may be necessary for bacterial multiplica-
tion, or again, even the oxygen may be required, as very energetic oxida-
tion must be taking place[1].

Table VIII. *Naphthalene* 34° C. *Cucumber soil*, 48 % *moisture*.

Grams of chemical added per kilo of dry soil		Millions of bacteria present per gram of dry soil after				Other organisms present after	
		6 days	19 days	28 days	74 days	6 days	28 days
Untreated		127·2	79·8	65·5	33·13	Fungi, eelworm	Fungi, eelworm
·05 % ($M/100$)	1·28 grams	516·0	117·4	94·6	35·3	Fungi, eelworm	Fungi, eelworm
·1 % ($M/50$)	2·56 ,,	1240·0	205·7	187·5	50·4	Fungi	Fungi
·5 % ($M/10$)	12·8 ,,	555·4	1384·0	939·7	89·19	Fungi cut down	Fungi cut down

Soil	Nitrate present after			Ammonia present after			Ammonia and nitrate present after		
	6 days	28 days	74 days	6 days	28 days	74 days	6 days	28 days	74 days
Untreated ...	576	850	1003	2·5	1·3	2·6	578·5	851	1006
·05 % ($M/100$)	578	863	1060	2·7	1·8	1·7	581·0	865	1062
·1 % ($M/50$)	432	697	922	32·7	2·0	1·6	465·0	699	924
·5 % ($M/10$)	386	525	858	67·8	2·4	2·6	454·0	528	861

Naphthalene has no effect on protozoa, eelworm and fungi at 18° C.
At $M/10$ there may be some slight effect on fungus growth and eelworm
numbers, but it is not easily recognisable. At 34° C. the compound is
more volatile and also more toxic to eelworm so that $M/10$ and $M/50$
both eliminate eelworm, while $M/10$ cuts down fungus growth appreciably.
Protozoa are not affected at any dose.

Exp. 2 (Fig. 3). $M/10$, $M/50$ and $M/100$ naphthalene were added to
cucumber soil of 48 per cent. moisture content, the bottles being closed
by cork stoppers. Since interchange of air was thus restricted, the
bacterial numbers rose more slowly than when cotton-wool was employed.

[1] Very often a decrease in bacterial numbers is accompanied by a rise in *nitrate* in the
control bottles —see phenol, and naphthalene. There are other cases where bacterial numbers
and nitrate rise together in the untreated soil, *e.g.* toluene, benzene and dichlorcresol.
Cymene was an interesting case, for in the untreated bottles a quick rise in bacterial
numbers was accompanied by a steep fall in nitrate, while later a fall in bacterial content
was reflected in a corresponding recovery in the nitrate value.

Reference to Fig. 3 shows that the numbers increased with the dose, a fall setting in after the 13th day. This fall slowed off considerably after the 34th day, and by the 74th day $M/100$ and $M/50$ were very little higher than the untreated soil. Yet $M/10$ had a population of 100 million bacteria per gram of dry soil above control even on the 247th day, and this occurring in cucumber soil where action goes on quickly makes it easy to accept and explain the Russell-Hutchinson rise of 40 million with toluene after 500 days in field soil where the process is always much slower. All smell of naphthalene was gone by the 13th day in the case of the two weaker doses, and by the 34th day when $M/10$ was added, while simultaneously the actual crystals ceased to be visible among the soil particles.

Table IX. *Naphthalene* 18° C. *Cucumber soil*, 48 % *moisture*.

Grams of chemical added per kilo of dry soil		Millions of bacteria present per gram of dry soil after			Other organisms present after		Protozoa present
		7 days	24 days	77 days	7 days	24 days	
Untreated	0 grams	167·3	163·1	104·3	Fungi, eelworm	Fungi, eelworm	F. C. Am.
$M/100$ ·05%	1·28 ,,	1817·0	229·2	168·7	,,	,,	,,
$M/50$ ·1%	2·56 ,,	4484·0	302·6	145·1	,,	,,	,,
$M/10$ ·5%	12·8 ,,	1912·0	759·0	303·8	Fungi reduced	,,	,,

Soil	Nitrate present after			Ammonia present after			Nitrate and ammonia present after		
	7 days	24 days	77 days	7 days	24 days	77 days	7 days	24 days	77 days
Untreated	580	569	648	2·9	2·4	2·2	583	571	650
$M/100$ ...	512	573	626	2·8	2·4	2·3	515	575	628
$M/50$ ...	462	562	578	2·8	2·4	2·0	465	564	580
$M/10$ ...	257	300	463	6·7	3·1	3·3	264	303	466

Exp. 3 (Table XII). As in the case of benzene, $M/10$ naphthalene was added to two lots of cucumber soil of 100 grams each in 1·5 litre flasks, and two similar controls were set up at the same time. All the flasks were hermetically sealed. They were opened on the 17th (Fig. 3) and 42nd days respectively, and in each case the numbers were practically at control level and naphthalene crystals could be seen in the soil.

The flasks opened on the 17th day were immediately closed with cotton wool plugs and counts were made on the 26th and 42nd days, that is, nine and 25 days after opening. In nine days the numbers rose to 3000 millions and then fell to 2600 millions on the 25th day after opening, and on the latter date no naphthalene could be detected by sight or smell.

The volatility of naphthalene is much less than that of benzene, and the fraction which can have escaped from the soil during the time that the flasks were sealed must have been negligible. It must therefore have been the lack of oxygen which checked the multiplication of the bacteria, unless the fact that carbon dioxide could not escape also acted as a check.

The requisite source of energy was present as naphthalene, but in the absence of oxygen the bacteria could not make use of it. It is interesting that they remained near control level. They may have been quiescent or, on the other hand, they may have been feeding on the normal, already partially oxidised, food supply.

Exp. 4 (Fig. 5). Field soil of 14·9 per cent. moisture was used for this, and the usual doses added. During the first eight days, no rise occurred at the $M/100$ dose, while a slight depression in numbers occurred at $M/10$ and $M/50$. By the 45th day however the numbers stood at 1000 and 870 million in the $M/100$ and $M/50$ bottles respectively, while $M/10$ stood at 530 million. The numbers with the highest dose went on rising steadily until the 196th day, when they stood at 1372 million, and would evidently rise still more because crystals of naphthalene were even then visible, and the smell fairly strong. All smell disappeared at the two weaker doses by the 45th day and from now until the 80th day there was a steady decline in numbers which continued, though at a much slower rate, between the 80th and 150th days.

All this evidence emphasises the much slower rate at which bacteria work in field soil, with its smaller water content and poorer aeration.

A very puzzling jump on the $M/10$ curve was found on the 17th day, but an explanation was soon found: the bottle had been opened twice and this allowed a fresh air supply, freer oxidation and utilisation of the energy so set free. Hence the line at $M/10$, between the 8th and 45th days should really follow the full line, and not the graph as it was first worked out.

Many see-saw irregularities in the curves throughout this paper are apparently caused by the difficulty of making up numerous sets of bottles in an identical manner and storing them under quite uniform conditions; the fit of the stopper, the amount of mixing with the chemical under investigation, etc., all have their disturbing effect.

The initial lag. $M/100$ naphthalene was added to two kinds of soil, and the bottles fitted with cotton-wool stoppers.

(*a*) Cucumber soil of 39·6 per cent. moisture. (*b*) Allotment soil of 16·4 per cent. moisture. Another experiment was carried out simultaneously,

in which $M/100$ naphthalene was added to field soil in bottles fitted with corks.

Mr Tattersfield, of Rothamsted, who was working independently on the subject, was in charge of (*a*) and (*b*) and made daily estimations of the disappearance of the naphthalene, bacterial counts being made simultaneously.

With cucumber soil there was a pause of 2–3 days, with allotment soil of 3–5 days and with field soil (Fig. 5) of 8 days before a very quick rise in bacterial numbers set in. This rise coincided in (*a*) and (*b*) with the disappearance of the smell of naphthalene, and with the time of quickest disappearance of the naphthalene, as determined quantitatively.

These interesting experiments rather indicate that the naphthalene first reacts with some substance present in the soil, possibly under the influence of an extra-cellular enzyme. When $M/10$ naphthalene was added to steamed cucumber soil (see later) the bacteria only reached 140 million in 13 days, against 1460 in normal soil, and this may be partly owing to interference with some compound in soil which first reacts with naphthalene.

After this possible first stage, oxidation of the naphthalene goes ahead, so that rapid disappearance of the chemical occurs simultaneously with quick rises in bacterial numbers.

Looking at matters from a different standpoint, the pause may be the time during which the bacteria are being trained to use this source of energy at various doses. It is interesting to note that $M/100$ gave 2420 million bacteria by the fifth day when a cotton wool stopper was used, and only 260 million when a cork was used.

Table X. *Naphthalene. Cucumber soil, 48 % moisture.*

Grams of chemical added per kilo of dry soil	Million bacteria present per gram of dry soil after							
	3 days	13 days	34 days	74 days	119 days	151 days	189 days	247 days
Untreated... ...	63·3	79·3	87·9	111·0	93·5	167·0	97·0	97·0
$M/100$ 1·28 grams	215·6	366·2	108·8	141·7	—	—	—	—·
$M/50$ 2·56 ,,	227·3	819·3	220·4	119·4	—	—	...	....
$M/10$ 12·8 ,,	260·2	1461·0	878·8	506·9	353·0	523·0	366·7	200·0

Table XI. *Field soil, 14·9 % moisture.*

Grams of chemical added per kilo of dry soil	Million bacteria present per gram of dry soil after						
	8 days	17 days	45 days	80 days	151 days	168 days	196 days
Untreated	69·9	69·9	61·7	53·5	62·4	48·8	48·8
$M/100$ 1·28 grams	68·7	257·6	1002·0	440·3	180·3	193·0	—
$M/50$ 2·56 ,,	46·9	384·1	870·2	399·4	437·1	292·0	. —
$M/10$ 12·8 ,,	54·7	1307·0	529·3	736·7	1190·0	1148·0	1372·0

Table XII. *Cucumber soil + M/10 naphthalene in sealed flask.*

	Sealed flask. Million bacteria present after		Opened flask. Million bacteria present after	
Dose	17 days	42 days	26 days	42 days
Untreated	177·8	82·2	157·0	104·0
M/10 ...	180·5	115·4	3094·0	2620·0

HEXANE. Formula C_6H_{14}. Molecular weight 86. Boiling point 71·5° C. Heat of combustion 992. Density ·63. Vapour pressure 97·4 mm. at 15° C
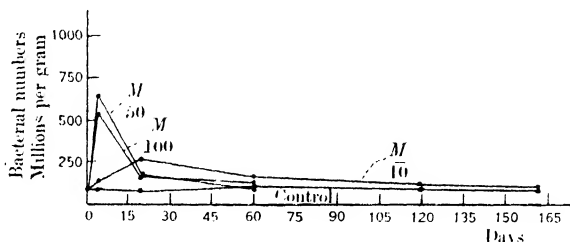
Fig. 6. Cucumber soil treated with hexane; 42 % moisture.
Numbers of bacteria per gram of dry soil.

Now that the two aromatic hydrocarbons benzene and naphthalene have been treated in detail, because they emphasise various points of importance with rather special clearness, it is interesting to describe the effect of an aliphatic hydrocarbon on cucumber and field soils.

M/100, M/50 and M/10 were added to cucumber soil, and rapid rises occurred in the case of the two weaker doses. By the fourth day the numbers stood at 460 and 550 million respectively above control, but a much slower rise occurred at M/10, where the numbers had only reached 187 million above control by the 19th day. On this date the numbers were again rapidly falling with the M/100 and M/50 doses, and no smell was left at any strength. By the 60th day the numbers had reached control again at the two weaker doses, and the temporary disturbance was apparently over (Table XIII).

However the bacteria at M/10 were still well above control level even on the 161st day, so that its effect lasts much longer. It is obvious from Fig. 6 that the maximum point of this curve has been missed owing to the long interval between counts—probably between the 19th and 60th days.

When these doses are added to field soil, a slight rise in bacterial numbers is seen by the eighth day, and this as usual proceeds more slowly than with cucumber soil. While the numbers have returned to normal by the 45th day with the M/100 dose, they are still well above control

at $M/50$ on the 81st day, and high but decreasing rapidly at $M/10$ on the 150th day. The smell is still persistent at $M/10$ on the 81st day, but gone by the 17th with the weaker doses. Thus hexane supports the evidence already presented that oxidation of hydrocarbons proceeds more slowly in field soils than in moist highly manured cucumber soils where the pore space is much greater and the surface exposed to air. The action of hexane on protozoa was not determined, but at the tested doses it has no action on fungi or eelworm.

Its effect on bacterial growth has been described in sufficient detail to make quite clear that the way bacteria oxidise this aliphatic hydrocarbon, and so avail themselves of a rather abnormal source of energy, rising temporarily to high numbers, in no way differs from the way soil bacteria utilise benzene and naphthalene and many other aromatic hydrocarbons. The difference is rather one of degree, since the bacteria cannot apparently rise to such high levels on the open chain aliphatic compounds as with the closed ring compounds like benzene, nor is any initial depression in numbers shown with hexane.

Table XIII. *Hexane. Cucumber soil, 42·3 % moisture.*

Weight of chemical added per kilo of dry soil	Million bacteria present per gram of dry soil after				
	4 days	19 days	60 days	119 days	161 days
Untreated... ...	90·5	72·6	102·1	89·4	74·4
$M/100$ 0·86 grams	546·7	155·7	133·0	—	—
$M/50$ 1·72 ,,	645·2	167·8	89·9	—	- -
$M/10$ 8·6 ,,	124·6	259·5	159·2	112·4	118·9

Table XIV. *Hexane. Field soil, 14·6 % moisture.*

Weight of chemical added per kilo of dry soil	Million bacteria present per gram of dry soil after				
	8 days	17 days	45 days	81 days	151 days
Untreated... ...	44·5	44·5	60·3	97·2	96·1
$M/100$ 0·86 grams	51·9	244·7	57·1	117·1	—
$M/50$	64·4	140·6	108·6	133·6	—
$M/10$	45·0	84·3	178·0	238·0	168·7

TOLUENE. Formula $C_6H_5CH_3$. Density ·88. Molecular weight 92. Vapour pressure 30 mm. at $15° C$. Heat of combustion 935. Boiling point $111° C$.

Preliminary experiment (Russell-Hutchinson method) (Fig. 8). Tomato soil of 36·9 per cent. moisture was used, and the soil aired after the reagent had been in five days. At the $M/100$ and $M/50$ doses a slight feeding effect was apparent, and by the 34th day the bacteria had risen some 50 million per gram of dry soil. This disturbance was over by the 105th day, the numbers having regained the untreated level. $M/10$ caused a depression of 14 million on the fifth day soon succeeded by

a non-sustained rise of 400 million per gram by the 34th day, these numbers falling again to 192 million by the 104th day.
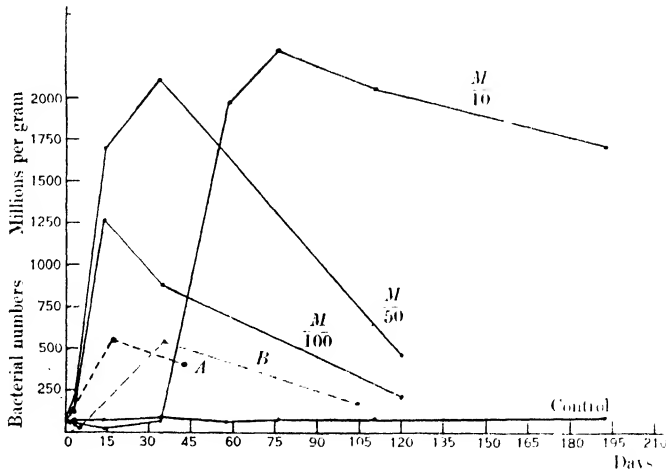
Fig. 7. Cucumber soil treated with toluene; 48 % moisture. Bacterial numbers per gram of dry soil. *A. M/50* in hermetically sealed vessel. *B. M/10* by Russell-Hutchinson method.

Ammonia (Fig. 9). A quick rise in ammonia occurs at all doses, and on the sixth day obviously rises with the dose. But although the bacterial numbers continue to rise at $M/50$ and $M/100$ until the 34th day, the ammonia drops again and is at the untreated level by the latter date,
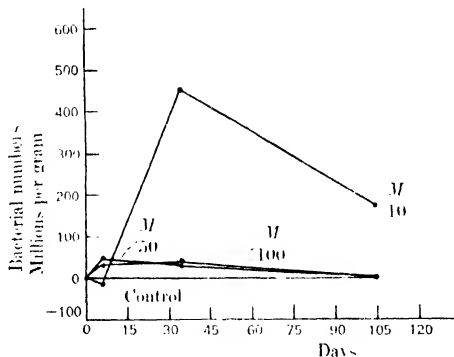
Fig. 8. Tomato soil treated with toluene; 37 % moisture. Russell-Hutchinson method. Difference of bacterial numbers from control, per gram of dry soil.

as though the nitrifying bacteria after temporary suppression, were again working. When the ammonia rises at $M/100$ and $M/50$ there is a simultaneous slight depression of nitrate. At the $M/10$ dose 132 parts of ammonia nitrogen have accumulated by the 34th day, and later it dwindles side

by side with the falling bacterial numbers, as though many of the abnormal number of bacteria were ammonifiers. More probably it indicates renewed activity of the nitrifiers as the toluene is destroyed. There is also at $M/10$ a considerable gain in nitrate from the sixth day onwards, and for the last ten weeks of the experiment the nitrate stands at 120 parts above control (Fig. 10). Hence the net result of adding $M/10$ toluene is a distinct gain in ammonia and nitrate over a period of 15 weeks, so that undoubtedly the toluene-eating bacteria elaborate readily available plant food, the increase being strikingly high compared with that given by other reagents. But whether such a method of ensuring a better crop could for one moment compete with the use of a cheap fertiliser, and so be of other than theoretical interest, is a questionable point.

The $M/10$ dose reduced the protozoa in number, yet some ciliates

Fig. 9. Tomato soil treated with toluene; 37 % moisture. Russell-Hutchinson method. Difference of ammoniacal nitrogen from control in dry soil.

and amoebae were still found, although the toluene had been six days in the soil before airing. In this respect then toluene is slightly less toxic
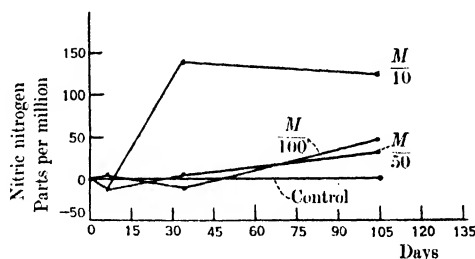
Fig. 10. Tomato soil treated with toluene; 37 % moisture. Russell-Hutchinson method. Difference of nitric nitrogen from control, in dry soil.

than benzene, where $M/10$ killed all but flagellates and some few small amoebae. Eelworm were cut down at $M/50$ and killed at $M/10$, the

latter dose also reducing the fungus growth to some extent. Hence the toxicity of benzene and toluene to larger soil organisms is somewhat similar, and quantitative tests at intermediate doses are necessary to discriminate between them.

Table XV. *Toluene. Tomato soil (Russell-Hutchinson method).*

Grams of chemical added per kilo of dry soil		Millions of bacteria present per gram of dry soil after			Other organisms present after			Protozoa present
		6 days	34 days	105 days	6 days	34 days	105 days	
Untreated	0 grams	41·2	90·9	18·76	Fungi, eelworm	Fungi, eelworm	Fungi, eelworm	F. C. Am.
M/100	0·92 ,,	72·0	149·1	20·99	,,	,,	,,	,,
M/50	1·84 ,,	85·2	127·2	19·56	Fungi	,,	Fungi	,,
M/10	9·2 ,,	27·2	544·4	192·4	Fungi cut down	Fungi cut down	Fungi cut down	F., few C. and few Am.

Soil	Nitrate present after			Ammonia present after			Nitrate and ammonia present after		
	6 days	34 days	105 days	6 days	34 days	105 days	6 days	34 days	105 days
Untreated	383	456	465	3·3	5·0	1·9	386·3	461·0	466·9
M/100 ...	385	445	509	9·3	8·2	1·9	394·3	453·2	510·9
M/50 ...	370	459	495	27·4	6·3	2·2	397·4	465·3	497·2
M/10 ...	370	596	586	34·0	137·0	4·0	404·0	733·0	590·0
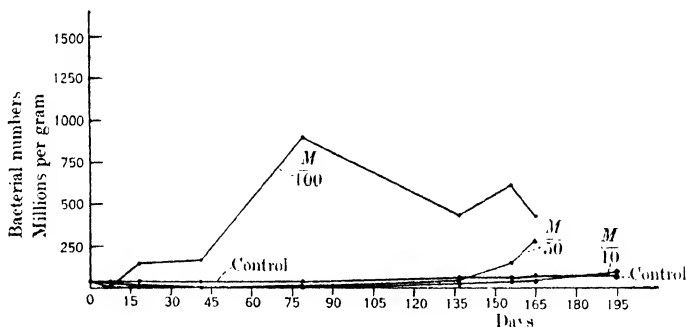
Fig. 11. Field soil treated with toluene; 14·6 % moisture.
Bacterial numbers per gram of dry soil.

Exp. 2. The action of $M/10$, $M/50$ and $M/100$ toluene on cucumber and field soils was next tested, the doses however being left in the soil to decompose. The corks were not sealed in the case of cucumber soil, but this was done for the field soil tests to prevent loss by volatility, and the latter are therefore slightly more satisfactory. Since benzene is so very volatile, probably the bacterial numbers obtained at the $M/50$ and $M/100$ doses with cucumber soil are far below what the whole dose would

have occasioned, had it not partly volatilised off. However toluene is much less volatile, so that the numbers will be nearer the true values. Since $M/10$ keeps the bacterial numbers down for 34 days, not at zero but at a level of 60 million per gram of dry soil, a rise setting in after this date, it seems probable that the 60 million bacteria which do tolerate the dose are reducing it until a concentration is reached which permits of a very rapid rise in numbers. As the graph of this rise is very similar to the curve for $M/50$, the reduced dose would seem to be nearer $M/50$ than $M/10$ (Fig. 7). Again, the bacteria may be learning to use the toluene as a source of energy, for hydrocarbons must surely be rather a novelty to them.

It is convenient here to consider in detail the course of events represented by the graph of bacterial growth occasioned by adding $M/100$ toluene to cucumber soil.

1. While the numbers are rising, most of the bacteria will be dividing, some may be quiescent and others dying, but the rapidly dividing ones screen all the rest and so the curve rises.

2. When the curve drops again, there is possibly still some of the new food left, besides the ordinary food supply and the supplementary food elaborated by these high numbers of bacteria. Yet the enormous population can no longer be supported, and so many die that the curve drops. Autotoxicity may possibly accelerate its fall. Many bacteria will be neither multiplying nor dying, but they are screened by the enormous number of deaths.

When $M/10$ pinene, which does not eliminate protozoa, is added to cucumber soil, the fall soon ceases and the curve remains horizontal over a long interval of time. The same thing is found with many reagents, the interval being of varying length. For some obscure reason a condition of equilibrium is temporarily established in such cases, the bacteria may be dying off as fast as they multiply, or may be in a quiescent state, but sooner or later the phase passes, and the decline in numbers again sets in.

The numbers at $M/50$ and $M/100$ toluene rise to 2000 and 1200 million respectively above control, before a fall sets in. On the 120th day these soils are again approaching control in bacterial content, so that the action is almost over. The rise which sets in on the 34th day at $M/10$ attains a maximum about the 77th day, and from then falls steadily, but the drop is so slow that even on the 192nd day, when the experiment was discontinued, the numbers had only fallen from 2400 million to 1700 million per gram of soil. At the two weaker doses all

smell of toluene disappears between the 3rd and 14th days, while an interesting point is the strong butyric acid-like smell which underlies the smell of toluene at the $M/10$ dose on the 34th day. This may be due to fermentation by anaerobic bacteria which tolerate the $M/10$ dose, as it is also noticed when $M/10$ benzene is added to cucumber soil in a sealed flask, and is of very short duration. All smell of toluene goes between the 34th and 58th days, by which time the bacteria have risen to 1968 million per gram.

Although the numbers in the $M/10$ soil were very high when the experiment stopped, analogy with the course of all other curves presented in this paper leaves no doubt that the falling bacterial content will slowly and inevitably return to control level. At least 6 to 12 months longer might be necessary for this return to equilibrium.

Two or three types of bacterial colonies are outstanding when the numbers are high at the two weaker doses, while a large number of brown streptococcus colonies is present when the $M/10$ curve is falling.

Effect of re-opening tested bottles. When $M/10$ series 2 was tested a second time, it was found that as early as the 26th day the numbers had risen to 60 million above control owing to the admission of fresh oxygen and a slight loss of toluene. Examining $M/10$ series 3 a second time on the 49th day the bacterial content stood at 300 million above control, a strong smell of toluene still remaining while a third examination on the 58th day demonstrated as many as 4710 million bacteria per gram, and all toluene smell was gone.

These experiments prove that additional air supply accelerates the rate of oxidation of toluene and consequent bacterial growth. A little of the dose may be unavoidably lost, but this is a comparatively unimportant factor.

On the 58th day counts were made of the bacteria in an $M/10$ bottle opened for the first time, and the numbers were only 1968 million against 4710 million in the thrice opened bottle, but the types of colony on the other hand were *far more numerous.*

Two types only gave the higher count, small white dots and the ubiquitous *liquefaciens* which probably turns up so often on account of its very resistant spore which survives treatment with all tested reagents. These observations strengthened a gradually growing opinion that enormous bacterial numbers are usually due to the special multiplication of one or two definite forms, and not due to increase of all soil bacteria. Probably there are no toluene-eating bacteria in the soil originally, but one or more forms can be induced to utilise it, and this

may hold for all reagents examined[1]. A fourth determination was made from $M/10$ series 3 on the 72nd day, and a third from $M/10$ series 2 on the 58th day. As was anticipated, the energy had been used up very rapidly, and the numbers were down again to 952 million and 461 million respectively. Hence in these repeatedly opened bottles the numbers are much nearer the untreated level by the 58th and 72nd days, than are the numbers in $M/10$ set 4 on the 192nd day, so that an inevitable return to the control numbers is clearly indicated for $M/10$.

It must not be forgotten that *all* determinations of the effect of $M/10$ after the 58th day, were made on an already opened bottle, no more sets being available. Yet repeated openings after this date did not lead to enormous rises such as occurred earlier, so that a free air supply is of greater significance during the early days of oxidation than later on in the experiment. Presumably all the toluene is broken down by the 58th day, and the subsequent changes are slow and perhaps incapable of much acceleration.

Effect of using a hermetically sealed flask (Fig. 7). The $M/50$ dose was mixed with 100 grams of cucumber soil and kept in a 1·5 litre flask. Two lots were put up, one of which was examined on the 17th and the other on the 42nd day. Some slow rise in bacterial numbers had occurred, but the comparatively low level attained (600 to 400 million) shows that a limited supply of oxygen hindered action, and the bacteria could not so quickly or fully avail themselves of the energy supply as in corked quart bottles. After the first count on the 17th day this flask was furnished with a cotton-wool stopper and the numbers at once rose, yet even on the 42nd day showed little signs of recovery from the initial check, unless a quick rise and fall had been missed between the two dates, as might easily happen.

Effect on protozoa. The accompanying table shows that although bacteria and protozoa may fluctuate inversely in numbers in the open field, protozoa are not to any appreciable extent concerned with the bacterial rises now under consideration. $M/50$ toluene occasions a rise of 900 million bacteria per gram more than the $M/100$ dose, and yet four times as many trophic amoebae were found at the higher dose as at $M/100$, while the reverse should be the case, were protozoa immediately concerned. Moreover the $M/10$ curve rises very little beyond the $M/50$,

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[1] At $M/100$ and $M/50$ benzene and toluene most of the soil bacteria can be found, many of them in greater abundance than on the control plates. It is at $M/10$ that enormous numbers of one or two kinds hide the rest, until the numbers are much decreased again. Then the characteristic soil bacteria are revealed once more.

yet $M/10$ eliminates all amoebae, and ciliates; these two results again being contrary to expectation.

Table XVI. *Cucumber soil. Protozoa[1] present after treatment with M/10, M/50 and M/100 toluene.*

	Control		M/100		M/50		M/10	
	Active	Spore	Active	Spore	Active	Spore	Active	Spore
Am.	11440	1760	2486	74	8376	74	0	26
C.	746	105	31	74	74	74	0	0
F.	?	25000	19500	5500	?	25000	19100	5500

On the other hand, by successive openings of the $M/10$ bottle one can vary the bacterial numbers from 1900 to 4710 million per gram, so that the cause of the high bacterial multiplication is undoubtedly reaction between toluene and oxygen, and independent of protozoa.

Action on field soil[2] (Fig. 11). Toluene acts much more drastically on field than on cucumber soil, but undoubtedly along the same lines. Experience enables one to interpret liberally and see the exact significance of the curves yielded over 195 days. All three doses cause an initial depression, and subsequent rise. At $M/100$ the rise is slow, reaching 900 million on the 78th day and falling again to 460 million by the 165th day. When the level reaches 900 million all smell has gone, and 100 days pass while the level drops by 440 million. Remembering that all drops in bacterial numbers discussed in this paper decrease greatly in rate as they near control, and that Russell and Hutchinson used a dose of 4 per cent. toluene[3], the oxidation of which would leave quite a large amount of residual products in the soil, after spreading out overnight, a content of 40 million above control even after 500 days is largely accounted for.

$M/50$ keeps the bacteria below control for 137 days, after which they rise by 210 million in the next 30 days, so that the rise had well begun when the experiment was discontinued. On this date also the smell of toluene had gone, such disappearance usually signifying the onset of a big rise in numbers. The smell persisted until the 156th–165th day at $M/10$ and the numbers well below control on the latter date soon began to rise, reaching 30 million above control by the 195th day when the experiment ended.

[1] The method used was in essentials that devised by Mr Cutler (15a).

[2] A diagram is usually given if the curve is complicated. If it represents a straight-forward rise and fall in numbers only one of a type is given.

[3] $M/10$ toluene $=0.9\%$. Therefore 4% —about $M/2$, a very large dose.

Effect of inserting cotton-wool stoppers instead of corks in M/50 and M/10 bottles, set 3, from the 50th–57th days. After substituting cotton-wool for cork stoppers for seven days in *M*/10 and *M*/50, set 3, bacterial estimations were made. As was expected, the numbers had risen, and stood at 150–200 million above control for about 60 days, whereas they were still well below the untreated level in the corked sets. All smell of toluene had disappeared in these bottles too. In the case of toluene the difference caused by reopening the same bottles, or allowing free interchange of air, is much less in field than in cucumber soil where change is always quicker.

Table XVII. *Toluene. Cucumber soil, 48 % moisture.*

Weight of chemical added per kilo of dry soil		Million bacteria present per gram of dry soil after						
		3 days	14 days	34 days	58 days	72 days	120 days	192 days
Untreated... ...		67·6	74·7	93·7	63·4	73·5	72·4	87
M/100	0·92 grams	111·9	1262·0	877·0	—	435·8	204·8	---
M/50	1·84 ,,	199·8	1697·0	2109·0	- ·	996·5	452·6	---
M/10	9·2 ,,	51·7	21·7	57·7	1968·0	—	2048·0	1705

Table XVIII. *Field soil, 14·6 % moisture.*

Soil	Million bacteria present per gram of dry soil after							
	7 days	18 days	41 days	79 days	137 days	156 days	165 days	195 days
Untreated	48·01	45·7	43·5	40·9	60·9	55·4	68·3	68·3
M/100 ...	10·2	147·9	167·8	899·9	440·3	604·0	431·0	—
M/50 ...	16·9	19·6	7·9	14·7	43·7	153·4	277·9	---
M/10 ...	9·4	12·1	10·9	11·2	62·4	37·5	39·8	99·8

Table XIX. *Toluene. Cucumber soil.*

Dose	Kept in sealed flasks after		Sealed flask opened on 17th day; after	
	17 days	42 days	26 days	42 days
Untreated	177·8	82·2	—	---
M/50 ...	665·8	418·2	1032	895

Table XX. *Million of bacteria, M/10 bottle, sets 2, 3 and 4. Cucumber soil, 58th day.*

Set 2 three times opened	...	461	millions
,, 3 ,, ,, ,,	...	4710	,,
,, 4 once opened	...	1968	,,

XYLENE. Di-methyl benzene. Formula $C_6H_4.CH_3CH_3$. Density 0·88. Molecular weight 106. Heat of combustion 1085. Boiling point 140° C.

When the action of *M/2*, *M/10* and *M/50* xylene on a tomato soil of 40 per cent. moisture is examined by the Russell-Hutchinson method,
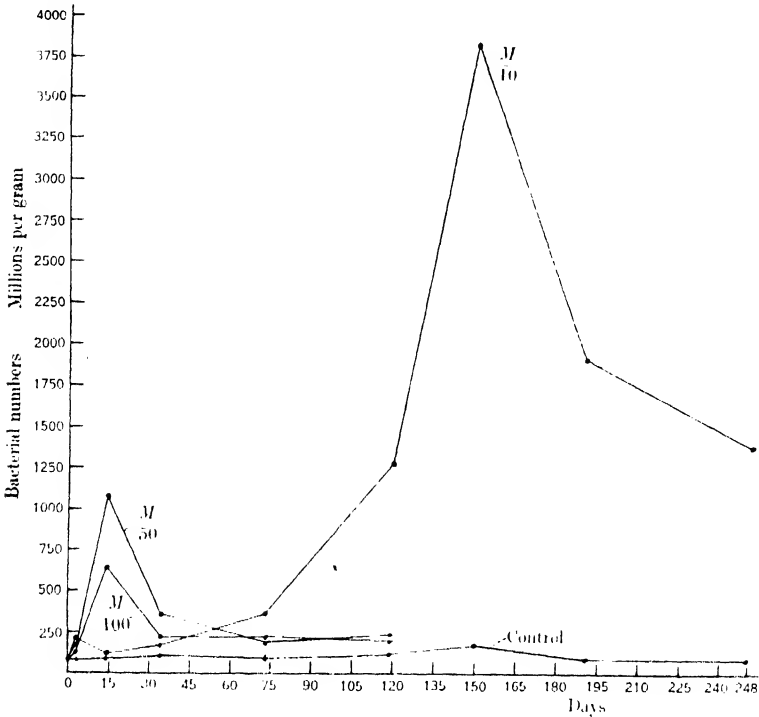
Fig. 12. Cucumber soil treated with xylene; 48 % moisture.
Bacterial numbers per gram of dry soil.
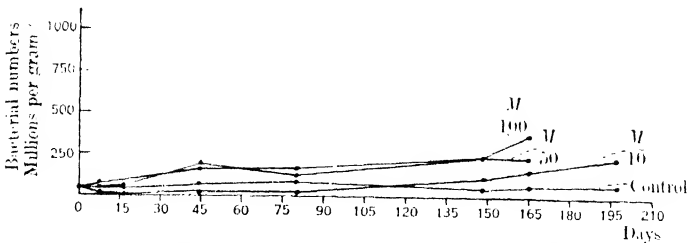
Fig. 13. Field soil treated with xylene; 14·6 % moisture.
Bacterial numbers per gram of dry soil.

an immediate feeding effect is registered at all doses, 525 million bacteria per gram being present on the 3rd day with the two higher doses and 27 million above control at *M/50*. However, the numbers are rapidly falling again on the 91st day, and stand at 315, 175 and 20 million

respectively above control in descending order of dose. *B. liquefaciens* is very prominent on all plates.

The following table gives the results of another similar experiment.

Table XXI. *Xylene. Tomato soil, 29 % moisture.*

Grams of chemical added per kilo of dry soil		Millions of bacteria present per gram of dry soil after			Other organisms present after			Protozoa present
		5 days	34 days	59 days	5 days	34 days	59 days	
Untreated	0 grams	44·1	56·8	—	Fungi, eelworm	Fungi, eelworm	—	F. C. Am.
M/50	2·12 „	67·5	83·6	138	Fungi	Fungi	Fungi	„
M/10	10·6 „	116·3	351·0	306	,	,.	Reduced fungi	„
M/2	53·0 „	106·3	297·5	—	„	„		„

Soil	Nitrate present after			Ammonia present after			Nitrate and ammonia present after		
	5 days	34 days	59 days	5 days	34 days	59 days	5 days	34 days	59 days
Untreated	688	629	—	5·4	7·6	—	693·4	636·6	—
M/50 ...	686	691	678	18·3	4·0	3·2	704·3	695·0	681·2
M/10 ...	574	597	677	23·4	98·5	126·3	597·4	695·5	803·3
M/2 ...	681	586	—	26·4	105·5	—	707·4	691·5	·· -

As with toluene and benzene, the ammonia content of the soil rises, and this can be detected even on the third day, the amount rising with the dose. It has gone again at M/50 on the 32nd day, but risen by 100 parts at M/2 and M/10, and by 106 parts at M/10 on the 57th day (tomato soil of 29·3 per cent. moisture). The nitrate has meanwhile risen by 60 parts at M/50, but on the other hand, great bacterial growth at M/10 and M/2 is reflected in a call on the nitrates, so that for the time being the soil is actually poorer in nitric nitrogen at these doses. Unfortunately, lack of sufficient data prevents one from deciding whether the net result of adding the various doses is a total gain in readily available nitrogen, or whether the accumulation of ammonia simply results from the death or temporary suppression of the nitrifying bacteria.

Qualitative tests showed no action on protozoa at any dose, so that the introduction of a second CH_3 group into the benzene ring lessens toxicity to protozoa. The M/50 dose eliminates eelworm, while even M/10 has but slight reducing effect on the growth of fungi, so that towards fungi also dimethyl benzene is less toxic than benzene and methyl benzene (toluene). Greater toxicity is however shown to eelworm, and it is possible that being less volatile than the two latter compounds it has more chance of attacking eelworm and their eggs. Such differences in volatility might easily screen the true relative toxicity of these three substances.

Exp. 2. $M/100$, $M/50$ and $M/10$ doses of xylene were left in corked quart bottles with cucumber soil of 48 per cent. moisture. The bacterial content rose rather rapidly with the two weaker doses, the rise beginning slowly and continuing much more quickly after the third day. $M/50$ gave 1000 million and $M/100$ 600 million by the 14th day, $M/50$ still smelling faintly of xylene but $M/100$ not at all (Fig. 12 and Table XXIII).

By the 34th day these numbers had fallen again to 350 and 220 million respectively, and all smell was gone. Now a further slow declining period set in, and even on the 120th day the numbers still exceeded the control by 100 million at each dose. Like hexane, at $M/10$ the rise was much slower, and had only reached 260 million above control on the 73rd day. Between this and 119th day the numbers rushed up and all smell disappeared. The crest of the wave of disturbance was reached on the 150th day, when the bacteria numbered 3816 million per gram. Then a decline set in and the numbers were 1906 million on the 190th day and 1366 on the 248th day, so that the drop is very gradual as in the case of toluene.

In *field soil* there was a similar sequence of events (Fig. 13). $M/10$ was much more drastic in action, reducing bacterial numbers below control for about 97 days when a rise set in. This attained about 150 million above control on the 197th day, while the smell of xylene was still very strong, so the rise would continue for some time. By the 80th day the $M/50$ and $M/100$ bottles still smelt of xylene, and the numbers had been steadily rising the whole time. $M/100$ stood at 300 million and $M/50$ at 160 million above control by the 165th day and the smell of xylene had gone. Fig 13 therefore demonstrates that action was proceeding so slowly that the crest of the wave of bacterial growth was hardly reached even with the weakest dose when the experiment was discontinued.

When free interchange of air was allowed at $M/10$ by substituting a cotton-wool stopper for a cork between the 50th and 57th days of the experiment, the rise in numbers above control set in earlier, reaching 90 million above control by the 57th day and 140 million by the 80th day.

Table XXII. *Xylene. Field soil,* 14·6 % *moisture.*

Grams of chemical added per kilo of dry soil	Million bacteria present per gram of dry soil on						
	7th day	16th day	44th day	80th day	148th day	165th day	197th day
Untreated	45·3	42·5	61·7	75·0	45	52·6	53
$M/100$ 1·06 grams	--	56·2	154·6	165·1	230	357·5	--
$M/50$ 2·12 ,,	--	46·9	180·3	125·3	230	211·0	--
$M/10$ 10·6 ,,	26·6	11·1	16·1	14·1	100	150·0	203

Table XXIII. *Xylene. Cucumber soil, 48 % moisture.*

Grams of chemical added per kilo of dry soil	Million bacteria present per gram of dry soil on							
	3 days	14 days	34 days	73 days	119 days	150 days	190 days	248 days
Untreated	83·3	94·6	102·5	92·2	107·5	165·4	97·0	97·0
$M/100$ ...	126·2	629·8	222·5	222·7	197·7	—	—	—
$M/50$ ...	161·3	1061·0	354·2	195·8	240·0	—	—	—
$M/10$ ...	202·1	110·0	166·5	359·0	1262·0	3816·0	1906·0	1366·0

FORMALDEHYDE (40 per cent. solution in water). Formula HCHO. Molecular weight 30. Heat of combustion 120–200.
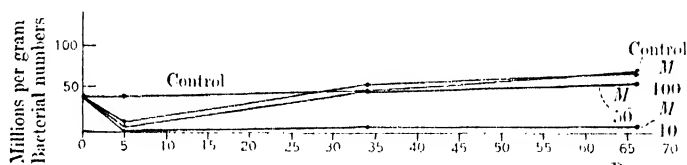
Fig. 14. Field soil treated with formaldehyde; 12·4 % moisture. Bacterial numbers per gram of dry soil.

Exp. 1. (Russell-Hutchinson Method.) Tomato soil of 33·3 per cent. moisture was used, the doses being $M/500$, $M/200$ and $M/50$. The highest dose visibly lessened the numbers of protozoa, but did not eliminate either amoebae or ciliates. Fungi and eelworm were not affected by these strengths, but in another experiment, a dose lying between $M/10$ and $M/2$ eliminated fungi, while $M/10$ killed all eelworm.

$M/50$ was the only dose which affected bacteria, and for a few days the numbers were below control level, but recovering about the 25th day they rose slightly above control and remained 10 million above this level for the next 60 days. Thus the protozoa were cut down at $M/50$, and the bacterial content somewhat raised. The ammonia content of the soil had risen 14 parts by the third day, while the bacteria fell and the protozoa were cut down. Presumably this ammonia is the result of a temporary pause in nitrification. By the 36th day the ammonia had gone, while the nitrate at $M/50$, after a slight initial fall, rose steadily between the 30th and 61st days of the experiment, possibly owing to an improvement in the physical condition of the soil.

Experiments carried out in pots and in commercial green-houses showed an improvement both in tomato plants and in the crop, when $M/50$ formaldehyde was applied (·06 per cent. of 40 per cent. solution). This result may also be owing to physical improvement of the soil, the reagent altering the colloids, as otherwise there appears no change (in

readily available nitrates or bacterial numbers) which would account for it. Formaldehyde is certainly effective against free living eelworm and fungus pests, but must be used cautiously as there is a certain cumulative effect if the ·06 per cent. dose is used year after year, so that the crop eventually falls. Perhaps the reserve food in the soil is drawn on too heavily and impoverishment follows, or the formaldehyde may gradually "fix" proteins and amino-acids and render them unattackable.

Exp. 2. When $M/10$ is added to field soil of 12·4 per cent. moisture in a corked bottle the bacteria are nearly all killed, and even on the 66th day the numbers stand at one million per gram against 70 million in the untreated soil, while the smell though faint is still present. Drastic reduction in numbers also occurs when $M/100$ and $M/50$ are used, but recovery follows by the 34th day, although in the next 30 days the control level is not passed (Fig. 14). Protozoa are all dead at the $M/50$ dose and only one or two amoebae and flagellates survive the $M/100$ dose, the tests applied being quantitative. The Partial Sterilisation theory cannot be expected to apply in this case owing to the probable "fixation" of the food supply. (Buddin also recorded death of all but flagellates at the $M/100$ dose without any corresponding rise in bacterial numbers in the case of allotment soil.)

The formaldehyde itself is too strong an antiseptic to serve as a food, so no increase of bacteria is to be expected. This point will be discussed again in a later part of the paper.

Exp. 1. Table XXIV. *Formaldehyde. Tomato soil, 33·3 % moisture.*
Russell-Hutchinson method.

Weight of chemical added per kilo of dry soil	Millions of bacteria present per gram of dry soil after		
	3 days	36 days	61 days
Untreated ...	64·7	86·6	55·9
$M/500$ 0·06 grams	74·2	55·7	69·5
$M/200$ 0·15 „	62·7	68·4	58·4
$M/50$ 0·6 „	18·8	96·8	66·0

Soil	Nitrate present after			Ammonia present after			Nitrate and ammonia present after		
	3 days	36 days	61 days	3 days	36 days	61 days	3 days	36 days	61 days
Untreated	569	601	601	2·7	2·5	1·2	571·7	603·5	602·2
$M/500$ ...	519	606	625	2·4	2·7	1·8	521·4	608·7	626·8
$M/200$ ...	549	632	600	1·8	2·2	1·4	550·8	634·2	601·4
$M/50$ ...	521	615	643	16·6	2·4	1·2	537·6	617·4	644·2

Exp. 2.   Table XXV.   *Formaldehyde.   Field soil, 12·4 % moisture.*

Weight of chemical added per kilo of dry soil	Millions of bacteria present per gram of dry soil after		
	5 days	34 days	66 days
Untreated ...	39·0	45·3	69·9
$M/100$ 0·3 grams	7·8	51·2	67·0
$M/50$ 0·6 ,,	4·3	43·7	53·3
$M/10$ 3·0 ,,	0·5	4·1	0·8

CARBON BISULPHIDE.   Formula CS_2.   Density 1·29.   Molecular weight 76.   Heat of combustion 265.
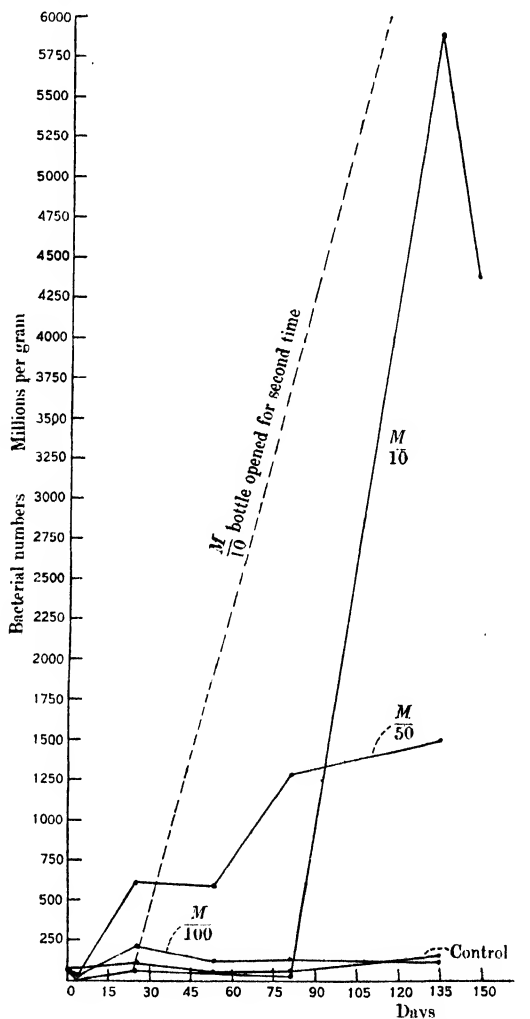
Fig. 15. Cucumber soil treated with carbon bisulphide; 42 % moisture.
Numbers of bacteria per gram of dry soil.

The usual $M/10$, $M/50$ and $M/100$ doses were added to cucumber soil of 42·3 per cent. moisture, and the stoppers sealed. It was not realised at the time that such sealing would hinder oxidation and slow down the course of the whole experiment compared with benzene and naphthalene, as well as check volatilisation.

The effect on protozoa was not tested, but $M/100$ killed eelworms and $M/10$ reduced fungus growth very considerably. An initial depression in bacterial numbers occurred at every dose, while the subsequent rise at $M/100$ was comparatively slight, the smell disappearing between the 53rd and 81st days and the numbers reaching 100 million above control by the latter date. However the bacteria rose much quicker and further at $M/100$ when the bottle was opened several times, so that doubtless the limited air supply checked the rate at which the reagent was oxidised. The bacteria at $M/50$ rose to 607 million and 1285 million by the 25th and 81st days respectively, the smell of carbon bisulphide still persisting. At $M/10$ the numbers remained depressed until the 25th day, but then rose to 1870 millions by the 53rd day (Fig. 15, broken line). It was confusing at first to find the numbers down again at 29 million in another bottle on the 81st day, and then rising rapidly a second time while the carbon bisulphide still smelled strongly, but the experiment with benzene in bottles provided with rubber stoppers explained this.

Table XXVI. *Carbon bisulphide. Cucumber soil, 42 % moisture.*

Grams of chemical added per kilo of dry soil	Millions of bacteria present per gram of dry soil after			
	4 days	25 days	53 days	81 days
Untreated... ...	71·5	106·8	50·9	55·4
$M/100$ 0·76 grams	33·2	204·6	117·1	147·5
$M/50$ 1·5 „	46·9	607·1	596·8	1285·0
$M/10$ 7·6 „	15·9	56·6	1869·0	29·4

Table XXVII. *Showing rapid rise of bacterial numbers in twice, thrice, and four times opened bottles.*

Soil	Series 2. Twice opened (after 31 days)	Series 2. Three times opened (after 53 days)	Series 2. Four times opened (after 81 days)	Series 3. Twice opened (after 148 days)
Untreated	95·3	54·8	59·3	83·9
$M/100$ ...	482·6	515·4	507·4	196·3
$M/50$ ...	622·7	873·6	1061·0	1073·0
$M/10$ ...	1843·0	4416·0	4379·0	8074·0

Soil	Series 4. Twice opened (after 134 days)	Series 4. Three times opened (after 148 days)
Untreated	153·9	83·9
$M/100$ ...	124·6	—
$M/50$ ...	1495·0	—
$M/10$ ...	5882·0	4373·0

The first high rise at $M/10$ was caused by a bad stopper which allowed quick interchange of air while the bottle showing 29 million bacteria per gram on the 81st day was fitted with a very well-sealed cork. The bottles of series A opened on the fourth day, were only half the size of the rest, and so the air space above the soil was much less, leading to very slow oxidation and delayed availability of the carbon bisulphide. Consequently in $M/10$ on the 147th day the bacteria had only reached 10 millions above control, whereas in the larger $M/10$ bottles they had risen to thousands of millions (8074 million per gram of dry soil). In the small bottle the reagent smelt very strong, but it was quite faint in the large bottle with very high numbers. The two accompanying tables emphasise the fact that the bacterial content of a treated bottle can be modified enormously by manipulating the air supply. The enormous numbers yielded by carbon bisulphide are remarkable, and are doubtless due to sulphur bacteria to a large extent.

PHENOL. Formula $C_6H_5.OH$. Molecular weight 94. Heat of combustion 732.
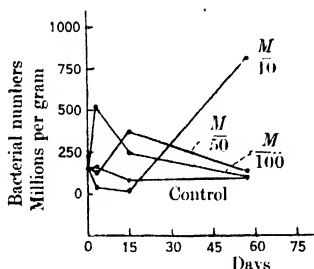
Fig. 16. Cucumber soil treated with phenol; 40 % moisture. Bacterial numbers per gram of dry soil.
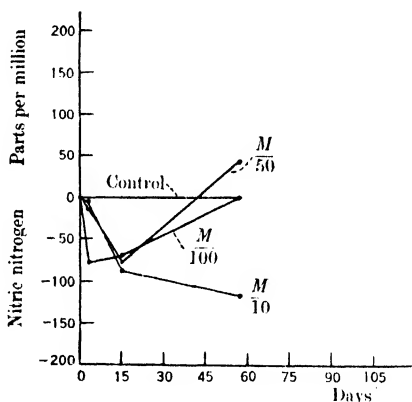
Fig. 17. Cucumber soil treated with phenol; 40 % moisture. Difference of nitric nitrogen from control in dry soil.

$M/100$, $M/50$ and $M/10$ doses were applied to cucumber soil of 40·14 per cent. moisture, the numbers rising immediately with the weakest dose (Fig. 16). Falling from 350 million above control on the third day, they had returned to control level on the 57th day when the experiment was discontinued. $M/50$ had also almost reached control again, after high rises succeeding a slight initial depression $M/10$ kept the bacteria below control for 20 days, but then they rose higher than with $M/50$ or $M/100$, and were still multiplying at the end of the experiment.

$M/10$ killed eelworm, reduced fungus growth to a trace, and among the protozoa cut down amoebae and killed ciliates, while $M/100$ and $M/50$ had no effect on these organisms. Nevertheless all three bacterial curves appear of one type (Fig. 16). Ammonia accumulates by the third day of the experiment, the amount rising with the dose. By the 15th day it has gone again at the two weaker doses, but steadily rising at $M/10$ reaches 57 parts by the end of the experiment. Hence nitrification is inhibited longest by the highest dose. Undoubtedly the nitrate store is temporarily depleted by the bacterial rises at all three doses, for it is diminished in ascending order of dose and in the order in which bacterial rises take place, reappearing as the numbers fall again (Fig. 17). This is comparable to the ordinary reducing action of carbohydrates in the soil. At $M/50$ there is an actual gain of 43 parts per million by the end of the experiment. Mr Sen Gupta, formerly of the Rothamsted Station, has published a paper describing his work on phenol. His quantitative determinations show rapid disappearance of phenol at the $M/200$ dose during the first two days, while Fig. 16 shows the bacterial numbers are simultaneously rising. Phenol-destroying organisms have been isolated from the soil by Mr Thornton and Mr Gray, of Rothamsted, all this evidence strengthening the argument that the high rises with various doses of phenol are due to the utilisation of the new source of energy by bacteria.

Table XXVIII. *Phenol. Cucumber soil, 40 % moisture.*

Grams of chemical added per kilo of dry soil			Millions of bacteria present per gram of dry soil after			Other organisms present after		Protozoa present
			3 days	15 days	57 days	3 days	15 days	
Untreated	0	grams	155·3	87·2	89·58	Fungi, eelworm	Fungi, eelworm	F. C. Am.
$M/100$	0·94	,,	504·7	249·9	95·5	,,	,,	,,
$M/50$	1·88	,,	137·4	362·1	118·8	,,	,,	,,
$M/10$	9·4	,,	43·85	22·61	800·5	Fungi	Trace fungus	F., few Am.

Soil	Nitrate present after			Ammonia present after			Nitrate and ammonia present after		
	3 days	15 days	57 days	3 days	15 days	57 days	3 days	15 days	57 days
Untreated	545	582	557	2·5	1·7	0·7	547·5	583·7	557·7
$M/100$ ...	469	512	559	3·6	2·0	0·0	472·6	514·0	559·0
$M/50$ ...	533	506	600	9·0	2·0	1·7	542·0	508·0	601·7
$M/10$ ...	542	494	440	16·5	38·7	56·2	558·5	532·7	496·2

The introduction of the OH group does not cause greatly increased stability such as occurs in the case of Cl and NO_2 groups, while the toxicity to eelworm is somewhat reduced. Again, benzene is more toxic

to nitrifying bacteria and 117 parts of ammonia accumulate at $M/10$, against 56 for phenol, but this comparison is not rigid as the benzene experiment was carried out by the Russell-Hutchinson method while the phenol experiment was not.

ORTHO CRESOL. Formula $C_6H_4.CH_3OH$. Molecular weight 108. Heat of combustion 884. Boiling point 200° C. Vapour pressure 1 mm. at 36° C.

The experiments were carried out on cucumber soil of 40·7 per cent. moisture in corked bottles and also by the Russell-Hutchinson method.

Table XXIX. *Ortho Cresol. Cucumber soil, 40·7 % moisture.*
Russell-Hutchinson method.

Grams of chemical added per kilo of dry soil	Millions of bacteria present per gram of dry soil after			Other organisms present after			Protozoa present
	4 days	10 days	38 days	4 days	10 days	38 days	
Untreated 0 grams	51	67·2	33·2	Fungi, eelworm	Fungi, eelworm	Fungi, eelworm	F. C. Am.
$M/50$ 2·16 ,,	135	626·6	230·0	,,	,,	,,	F. C. ,,
$M/10$ 10·8 ,,	30	16·8	204·7	Fungi	Less fungi	Fungi (trace)	(Am. modified)
$M/2$ 54·0 ,,	45	16·8	14·9				

Ammonia present after 42 days: (1) Control and $M/50$, 4 parts per million.
(2) $M/10$, 50 parts per million.

The bacteria rose quickly when $M/50$ cresol was added to soil, and the smell soon disappeared. By the 38th day the numbers were rapidly decreasing at this dose, and the graph resembled that of phenol. The numbers at $M/10$ and $M/2$ suffered an initial depression, a quick rise setting in at the former dose on the 17th day while at $M/2$ the numbers were still below normal at the end of the experiment. Still, as they were undoubtedly rising to control level, a rapid increase would probably follow later. In a second experiment where the soils were not spread out, $M/50$, $M/10$ and $M/2$ were added to a tomato soil of 30 per cent. moisture and the smell of cresol was still strong at $M/2$ after 280 days, the numbers remaining well below control level. However at $M/50$ and $M/10$ all smell was gone and the bacteria had regained control level.

The inhibition of nitrification lasts longer at $M/10$ than at weaker doses, so that on the 42nd day the ammonia content of the $M/10$ soil stood at 46 parts above control and $M/50$. $M/10$ kills eelworm, cuts down protozoa and reduces fungus growth to a trace. $M/2$ apparently kills all soil organisms, but as it persists so long in the soil some may only be inhibited.

As the toxic value of cresol[1] is thus very similar to that of phenol[2] it is preferred in commercial glasshouse work on account of its comparative cheapness. The usual dose is $M/50$ or ·25 per cent. and as this is speedily destroyed in soil, the pests it kills must be wiped out soon after treatment. "Club" is not cured but only very slightly cut down by the ·25 per cent. dose and larger doses cannot be used, as they remain long enough to injure the crop.

Part II.

This section of the paper deals with generalisations concerning the behaviour of the series of aromatic hydrocarbons already described. It considers in turn the effect on bacteria, ammonia and nitrate.

Examination of gelatin plates shows that the whole series appear to be attacked by the same or similar species of bacteria. When the *early* history of the rises given by $M/10$ is studied, and all the values plotted on one graph (Fig. 18) it is found that whether the experiments are conducted by the Russell-Hutchinson method or not, the series of lines representing bacterial rises lie according to the sequence of molecular weight and heat of combustion.

Table XXX. *Aromatic hydrocarbons. Cucumber soil, about 40 % moisture.*

Names	Molecular weight	Heat of combustion	Position on graph in rising series
Benzene ...	78	800	1
Toluene ...	92	935	2
Xylene ...	106	1085	3
Pseudocumene	120	?	4
Mesitylene ...	120	1252	5
Naphthalene	128	1235	6
Cymene ...	134	1414	7
Pinene ...	136	1489	8

This is all the more striking in the experiments carried out by the Russell-Hutchinson method since this part of the work was spread over two years, and yet the sequence held, despite the facts that the soils varied in source and moisture and that the intervals between counts were arbitrary and haphazard instead of uniform throughout. Such

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[1] The OH group added to toluene gives cresol which is rather less toxic to eelworms and protozoa. Thus toluene $M/50$ cuts down eelworm but $M/10$ cresol is the smallest dose to affect them, and while $M/10$ toluene kills all protozoa but flagellates, some amoebae and ciliates survive $M/10$ cresol.

[2] The toxic value of phenol and cresol is about the same; as it is for benzene and toluene.

details were carefully studied for the second series of experiments, but the results merely confirmed the first. The sequence becomes obscured later on in the experiments owing no doubt to volatility, secondary bacterial changes initiated by the early rise already discussed, and to the accelerated decomposition of organic matter in these soils.
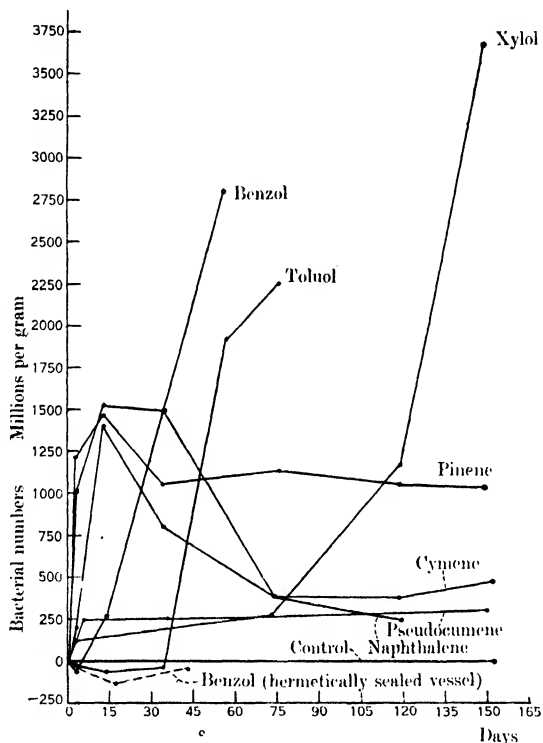
Fig. 18. Soil treated with $M/10$ aromatic hydrocarbons. Difference of bacterial numbers from control per gram of dry soil.

Arranging the rises at $M/50$ and $M/100$ on similar common graphs confirms the sequence shown by $M/10$, but with the smaller doses volatility will of course be a more potent disturbing factor, and the lines representing rises caused by the more volatile substance like benzene and toluene move out of their true place. This would tend to obscure the sequence were not $M/10$ a guide. The sequence also holds for these doses in field soil, as regards the early part of the experiments, but later on is obscured by volatility and other factors. Even though the numbers are kept below control for at least 60–80 days by $M/10$ benzene, toluene and xylene in field soils, the sequence is there. Therefore, as the sequence always holds,

it proves that the rises are caused almost entirely by the actual reagent added.

The agreement between the rises and the molecular weight and heat of combustion fails, as is natural when comparison is attempted between dissimilar substances, *e.g.* carbon bisulphide and benzene. The bacteria plated are as a rule very different in two such cases. The heat of combustion of carbon bisulphide is low—only 265, yet the bacterial numbers at $M/10$ reach 8000 million per gram.

When the ammonia values for the same series at $M/10$ (Russell-Hutchinson method) are likewise plotted on a common graph, the sequence holds (Fig. 19). But now benzene, toluene and xylene stand highest, pseudocumene and mesitylene[1] are still intermediate while pinene, cymene and naphthalene lie alongside control as they produce practically no ammonia. This ammonia may serve as an index of the relative toxicity of the series to nitrifying bacteria, or perhaps of ammonifying activity among the heightened population and in the former and more probable case the drop will commence when the nitrifiers revive. This will take place when the reagent is largely decomposed and when the numbers are falling again, and indicates that suspension of activity rather than death of the nitrifiers probably occurred in all the cases of Partial Sterilisation quoted by Russell and Hutchinson.

Fig. 19. Soil treated with $M/10$ aromatic hydrocarbons. Difference of ammoniacal nitrogen from control in dry soil.

When the nitrate (Fig. 20) and nitrate + ammonia values yielded by the aromatic hydrocarbons at $M/10$ are plotted on common graphs no sequence is shown. Pinene giving an immediate rise of 700 million bacteria causes only a slight depression in the nitrate level, while

[1] $M/10$ Pseudocumene and mesitylene yield higher numbers by the third day than do toluene, benzene and xylene, and with this goes a quicker rise in ammonia. Ultimately, however, more ammonia accumulates with the three latter substances.

naphthalene[1] with 1700 million shows a huge temporary fall. Again toluene, with a gradual rise to 450 million bacteria, yields the highest increase in nitrate value in the series, while xylene with 116–350 million

Table XXXI.

Substance M/10 dose	No. of million bacteria above control on 3rd day	Parts per million of ammonia above control on 3rd day	Highest value for ammonia
Pseudocumene	28·6	28·6	60
Mesitylene ...	84·0	28·9	70
Xylene ...	72·0 (5th day)	18·0	122
Toluene ...	–14·0 (5th day)	30·7	140
Benzene ...	–48·5	15·8	120
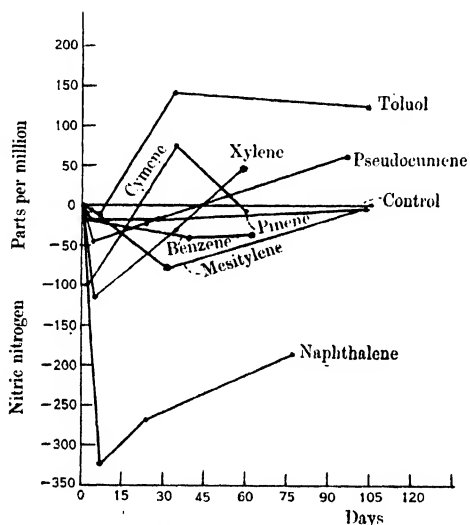
Fig. 20. Soil treated with *M*/10 aromatic hydrocarbons. Difference of nitric nitrogen from control in dry soil.

causes an initial depression. Obviously such variations do not go hand in hand with numbers, so they must be governed by the activity of the particular species of bacteria the reagent calls into prominence.

[1] This fall occurs also when carbohydrates are added to soil (13), and with phenol, xylene and cymene.

PART III.

It is clear from the detailed description of the action of the various reagents that all the bacterial rises they cause are temporary and that sooner or later the numbers will return to control level. In field soil the disturbance may easily be extended over 400–500 days, as the action is very slow.

Six experiments were made to determine whether these rises could take place in soil which did not contain any protozoa, and all the results agreed in proving this possible.

Exp. 1 (Fig. 21). Rises produced in steamed soil, where the protozoa had been eliminated. Three lots of steamed soil were treated:

A. Cucumber soil steamed 16 days before for four hours.

B. Cucumber soil steamed 68 days before for four hours.

C. Field soil steamed 68 days before for four hours.

Quantitative determinations showed complete absence of protozoa[1] (in steamed field soil 12 flagellates per gram were found). Yet no great rise in bacteria had been found during the interval of 68 days since *B* and *C* were steamed. This seemed strange, for steaming is regarded as a classic method of ensuring Partial Sterilisation, and yet it was followed by comparatively poor rises[2]. The rises quoted by Russell and Hutchinson for heat at 65° C. were next plotted, and also found very small compared with the results of adding toluene or carbon bisulphide to cucumber soils. There was just a possibility that some inhibitive toxin had been elaborated by steaming and that its presence might be interfering with the expected rise in numbers. Yet at this point in the work the view taken was that such rises as occurred were caused by the extra food set free on steaming, and that the bacteria could not rise further because there was no energy supply to rise on. Therefore $M/10$ naphthalene, $M/100$ toluene, and $M/50$ benzene were added to all three soils, an untreated bottle remaining for comparison. ($M/100$ benzene was used for field soil.) The numbers showed distinct rises in every case (see Table XXXII), while the smell of the antiseptic gradually died away. The rises followed a course quite comparable with those found in normal soil when the reagents are added, but the rate of action was much slower.

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[1] See footnote (3), p. 29.

[2] Cucumber soils were steamed for four hours on various occasions and the protozoa were always killed, while the subsequent bacterial rise varied suprisingly in different cases. In one sample the numbers rose rapidly to 700 million above control by the 18th day, but they returned to 30 million above control on the 122nd day, when the extra food rendered available by steaming was finished.

This is not surprising, as the population was simpler, and it might happen that some important factor in the soil which starts decomposition was materially weakened by steaming. As an example naphthalene had only given a rise of 150 million in 13 days against 1300 million in similar unsteamed soil.
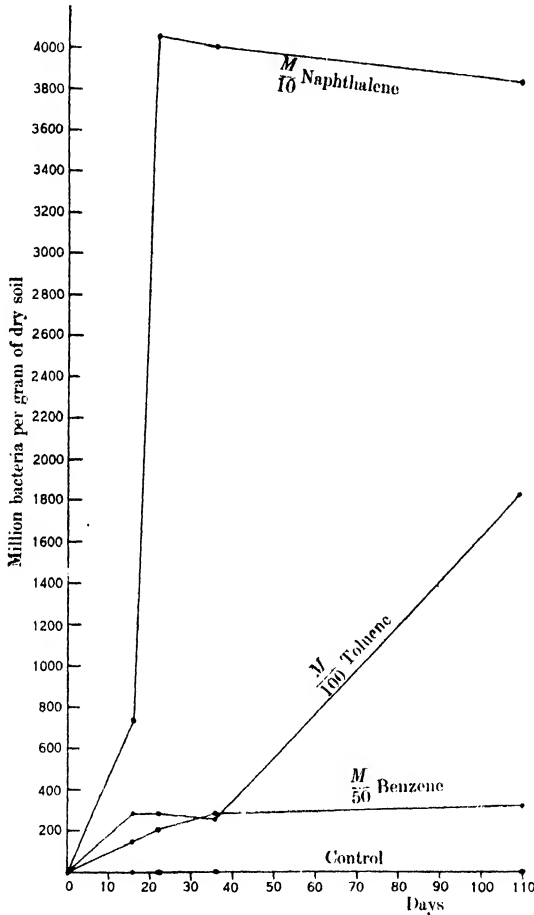
Fig. 21. Steamed cucumber soil free from protozoa treated with naphthalene, toluene and benzene. Increase of bacterial numbers per gram of dry soil.

It would be strange if three reagents chosen at random were all capable of neutralising a toxin which was repressing bacterial increase so effectively that the rise to be expected after steaming was not manifested in 68 days.

It is simpler to believe that the bacteria failed to rise because there was no energy supply, and that they responded as soon as it was added, such response being independent of any action on protozoa.

Table XXXII. *Rises in steamed soil; millions per gram of dry soil.*

No. of count	Days since soil steamed	Days since chemical added	Kind of soil	Steamed only	+ Naph. $M/10$	+ Toluene $M/100$	+ Benzene $M/50$
1	81	13	"Old" Cu. Chemicals	107	252	119	114
2		21	added 68 days after	106	1418	178	189
3		35	steaming	129	1270	115	454
4		106		108	4343	218	1490
5		128		244	—	466	—
1	32	16	"New" Cu. Chemicals	120	867	405	272
2		22	added 16 days after	120	4188	403	318
3		36	steaming	120	4134	391	401
4		110		120	3967	1932	448·7
5		129		120	—	24,100	—
1	81	22	"Old" Field. Chemi-	29	53	96	104
2		34	cals added 68 days after	23	62	71	107
3		105	steaming	12	37·7	68	94·5

The last three of benzene are $M/100$.

Although the rate of rise is slowed down in steamed cucumber soil, the numbers eventually reached in no way fall short of those attained in unsteamed soil.

The greatest slowing down occurs with field soil, the rises with naphthalene toluene, and benzene reaching only 26, 70 and 80 millions respectively during the 105 days of the experiment.
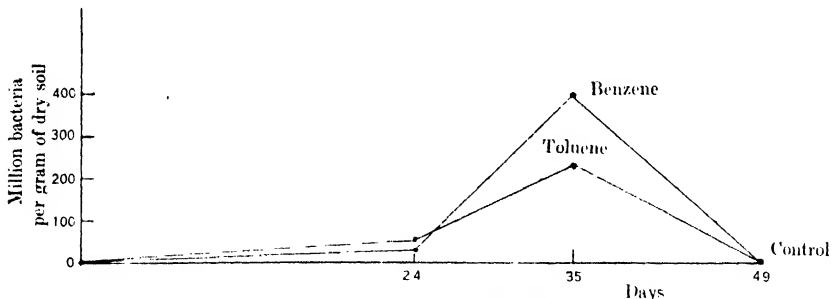
Fig. 22. Soil bottled in year 1846 now treated with $M/100$ benzene and $M/100$ toluene. Increase of bacterial numbers per gram of dry soil.

Exp. 2 (Fig. 22). Broadbalk soil was used which was bottled off in 1846, and dried down to 3 per cent. moisture in a warm oven in 1860. Complete absence of protozoa(15a) was determined by quantitative tests on this sample. The moisture was bought up to 12 per cent. with sterile water, so that bacterial multiplication might proceed at a reasonable rate. Three bottles were set up: (*a*) untreated; (*b*) $M/100$ benzene added;

(c) $M/100$ toluene added. These were corked and sealed. Estimations of the bacteria were made from time to time, a difference arising between the untreated and treated samples. After a period of increase the numbers returned to control level. Thus, when toluene and benzene are added to field soil which *contains no protozoa*, the usual temporary wave of disturbance affects the bacteria. They increase for a time in number, but gradually fall back to the untreated level. A faint cloudy spot was the prevailing type of colony on the untreated plates, but an opaque white disc was more prominent in the others. The bacteria present in the dry soil must possess very resistant spores, to withstand years of desiccation. The gelatin plates showed rather different colonies from those characteristic of a normal field soil. There were 8·4 million bacteria per gram of dry soil at 3 per cent. moisture, but after standing 21 days at 12 per cent. the numbers rose to 394 million, and to 831 million by the 43rd day. This number, 10–20 times as high as the normal content of field soil, indicates an abnormal supply of food resulting in some way from long desiccation. Nevertheless the bacteria avail themselves of a still further source of energy when benzene and toluene are added, independently of the presence or absence of protozoa.

Table XXXIII. *Bacterial rises in* 1846 *soil.*

No. of count	Days after adding chemical		Date	Control	Benzene	Toluene
1	(Benzene and toluene both smell strong)	24	19 Nov.	831 mill.	859 mill.	874 mill.
2	(Toluene strong smell, benzene fainter smell)	35	30 Nov.	797·5	1186	1026
3	(Both have weak smell)	49	14 Dec.	980·4	950·8	915·6

Exp. 3 (Fig. 23). Soil recovering from treatment with $M/10$ toluene was *re-treated* with $M/50$ toluene. The numbers rushed up again even quicker than when $M/50$ was added to an untreated soil[1]. The effect would have been even more striking had the numbers in the $M/10$ soil returned approximately to control level before re-treatment, but time did not permit of this, so the $M/10$ bottle was taken from three different sets, the numbers standing at 2700, 1079, and 380 million respectively. After six days in the first case and 10 days in the other two all smell of the newly added toluene was gone while the bacterial contents had increased to 4942, 2945 and 1523 million, or risen by 2242, 1865, and 1143 million respectively; rises which obviously increase with the number

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[1] The acceleration in speed occurs because the bacteria are already trained to use the substance, and because the proportion of trained bacteria in these soils is very high. Mr Tattersfield and Mr Thornton of Rothamsted have shown similar accelerated rises when further doses of naphthalene and phenol are added to previously treated soil.

of bacteria initially present. The numbers in untreated soil increase by 1200 million only during the first 14 days after adding $M/50$ toluene.

As $M/10$ toluene kills all protozoa save a few flagellates (quantitative tests[15a] on the 165th day after adding proved this) the new $M/50$ dose cannot affect them, so the second rise is obviously owing to the utilisation of toluene and independent of protozoa.

This is a third case of huge rises following the addition of toluene to a soil whence protozoa have already been removed, all three instances indicating the toluene itself as the source of energy.

Exp. 4 (Fig. 24). A cucumber soil was treated with $M/50$ chlorpicrin which killed all the protozoa except a few flagellates (this was proved by quantitative tests). By the 13th day the bacteria were reduced by 50 million but by the 57th day they had returned to control level, at which they approximately remained for the next 80 days, the numbers of species being reduced at the same time. Treatment with other reagents equally fatal to protozoa, such as toluene, results in a great increase of bacteria above control. The failure of chlorpicrin to produce the same result might be due in the first place to a toxic dose of this reagent remaining in the soil. This is improbable, as the number of bacteria is about the same as in the untreated soil, and it could not be detected by its odour, which is extremely penetrating. The obvious explanation is that the difference is due to

Fig. 23. Cucumber soil treated with toluene; 40 % moisture. Increases of bacterial numbers per gram of dry soil caused by (broken line) one dose of $M/50$; (full line) one dose of $M/50$ following treatment with $M/10$.

the very low food value of chlorpicrin, the larger part of which consists of unoxidisable substances. The bacteria have not risen because no extra energy has been supplied. (The slight rise shown is attributable

to food made available by chemical action.) Toluene $M/50$ was added to this soil, and six days afterwards the numbers had risen by 43 million, 33 days later by 211 million and 23 days later still by 400 million. There was no smell of the reagent on the 11th day after it was added. The results were confirmed by a second experiment which showed a rise of 425 million by the 11th day and 618 million 17 days later still. Therefore
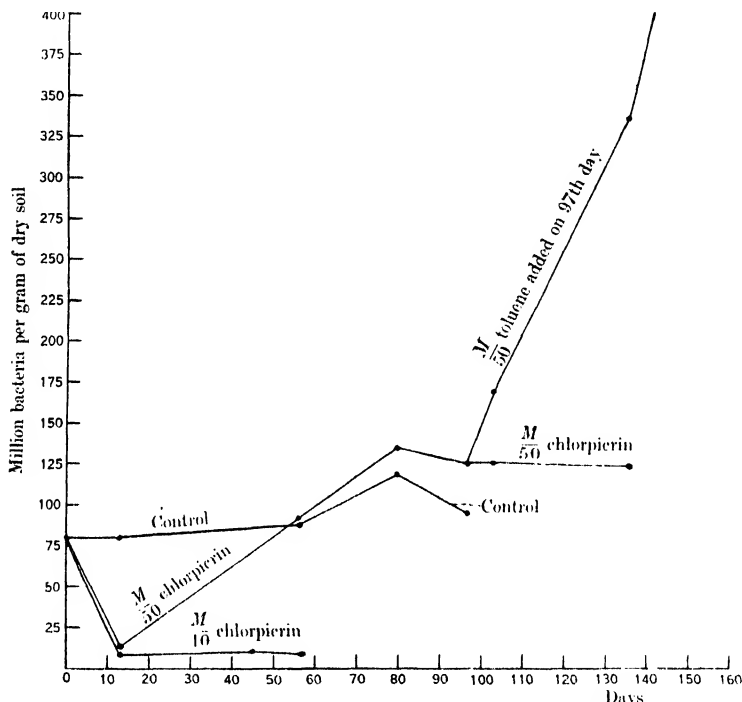
Fig. 24. Cucumber soil treated with chlorpicrin and later with toluene; 46 % moisture. Bacterial numbers per gram of dry soil.

even the simplified population left after treatment with chlorpicrin makes use of energy supplied as toluene, and the numbers rise rapidly independently of the presence or absence of protozoa.

Exp. 5. $M/50$ toluene was next added to cucumber soil that had almost recovered from treatment with $M/50$ chlordinitrobenzene. Again the population was simplified, but the very high numbers it had attained had not quite returned to normal. Time did not permit of longer waiting, so quantitative tests were made which proved the absence of protozoa, and toluene was then added. For 39 days the smell of the reagent remained very strong, and no rise in numbers took place, but by the 62nd day the smell was gone and the numbers had risen by 100 million

per gram, a rise which continued steadily for 34 more days when the experiment was discontinued.

This is another case of utilisation of toluene by the profoundly modified population of a soil from which the protozoa had been eliminated before the beginning of the experiment.

Exp. 6. (Figs. 14 and 25.) A final experiment was made with two bottles of field soil, previously treated with $M/100$ and $M/50$ formaldehyde respectively. Quantitative tests proved that all protozoa were dead at $M/50$ and only a very few flagellates and a few amoebae left at $M/100$. When formaldehyde was added an initial depression in bacteria occurred, but they had returned to normal by the 34th day, and did not rise further during the next 33 days. As has been pointed out, formaldehyde is an antiseptic which could have at best only a low food value, and has the power of reducing the food supply already present, so no rise is to be expected. It was unlikely that any formaldehyde still persisted as there was no smell and the numbers had been normal for 33 days. $M/100$ toluene was added to these soils, and some days later a rise was found. In the $M/100$ bottle it was appreciable on

Fig. 25. Field soil partially sterilised with $M/100$ formaldehyde; 12·4 % moisture. Increase of bacterial numbers per gram of dry soil caused by adding energy in the form of $M/100$ toluene.

the 10th day, but at $M/50$ it was delayed until the 14th day. The numbers on the 16th day stood at 480 million above control in the $M/100$ bottle, and at 140 million above control on the 25th day with $M/50$. Again rises follow when energy is supplied, and this negatives the idea of any persistent toxic effect due to unaltered formaldehyde.

General Discussion.

The assumption that the sequence graphed in Fig. 18 is due to the differences of the heats of combustion requires justification. The sequence is also shown if molecular weight be plotted instead of heats, and since the doses vary as the molecular weights it is possible that the effect is due to the weight of food supplied irrespective of its chemical nature. The following considerations may help to a decision.

If soil be treated with $M/10$ doses of benzene and its mono-, di-, and trimethyl derivatives toluene, xylene and pseudocumene there is at first a lag, and then the rapid rise sets in on or after the 3rd, 33rd, 75th and 151st days respectively. The exact days cannot be determined, but it may safely be said that the range is of the order of one to ten. Now the range of molecular weights and doses is of the order of one to one and a half only, and we may conclude that the difference of lag is due to the chemical difference of these closely related substances and not to the dose.

Again, in the assimilation of aromatic compounds the most difficult step is probably the breaking of the ring; to quote an analogous case, it has been found, by experiments not described here, that cyclohexane is much less readily attacked than hexane. Now for each ring broken up benzene gives 800 units of energy, but pinene 1489, *i.e.* nearly 90 per cent. more. Pinene is therefore to be expected to provide energy more easily than benzene.

Finally, if the actual percentage weight of the dose is of much importance we should expect to find that, on the average, varying doses of one substance added to soil should produce effects roughly proportional to their weights; exact agreement cannot be expected, even assuming perfect counts, owing to the varying antiseptic effect of different doses on a mixed population. Actually we find that only two of the experiments show such a proportionality.

It is therefore suggested for the present that for closely related substances it is the heat of combustion, *i.e.* the energy available to bacteria, which is the deciding factor.

Large rises follow the treatment of soil with substances, such as lime, which are not foods and do not destroy protozoa at the doses in question; the effect is plainly due to the food produced by the action of the reagent on the soil. Steaming also causes rises, and though it kills protozoa the shape of the curve suggests strongly that there is also a feeding effect, as is to be expected.

Russell and Hutchinson showed that some of the substances discussed here caused a marked increase in fertility; in view of the experiments described this must be ascribed in large part to the rapid breaking down of the soil by the increased number of bacteria. The present high cost of such chemical reagents renders it improbable that they will be able to compete with the cheap commercial fertilisers except when they also destroy pests like eelworm and wireworm.

The question whether they really eliminate eelworm and fungus

spores, or simply inhibit them so long as they are not decomposed by the soil bacteria, is a most important one, and substances which suggest themselves for investigation are cresol, chlorphenol, chlordinitrobenzene, etc. An ideal pest killer would be one which lasted in the soil only long enough to do its work and then was decomposed by bacteria. During early trials it was found that doses fatal to the pests were fatal to the crops also, and doses tolerated by the crops spared the pest.

The use of gelatine plates for counts in the presence of liquefying bacteria is open to criticism. At the time that the work was begun there was no better method known, and though the gelatine-agar plate was introduced by Mr Thornton while the work was still in progress, it did not appear to be advisable to change the technique so radically at that point. The use of gelatine has at least the advantage of making the work comparable with by far the larger part of earlier investigations dealing with soil bacteria.

The method employed in the presence of liquefiers was as follows. Three plates were prepared for each count, and if liquefaction occurred only such segments were counted as were perfectly dry, and the numbers corrected accordingly. If the liquefaction was serious the experiment was repeated. In cases where there was reason to suspect the presence of liquefiers a count was made on the first day, so as to obtain at least a minimum number as a guide.

Fortunately the rises observed were so large that very serious errors in the counts would have made no difference to the conclusions drawn. The general agreement of the curves is a strong argument for their accuracy as guides to the general course of events.

Lastly, the large number of counts made as controls on untreated soils failed to show under the conditions of the experiments any great sawedged curve such as Mr Cutler has proved for soils under field conditions. Maximum and minimum counts stand at 270 and 30 millions for cucumber soil, at 168 and 16 millions for tomato soil, and at 111 and 37 millions in field soil, with means of 90, 90 and 60 respectively.

SUMMARY.

1. Quantitative determinations have been made of the effect on soil protozoa and bacteria of various antiseptic substances, including benzene and its homologues and derivatives, carbon disulphide, ammonia, formaldehyde and chlorpicrin. Ammonia and nitrates were determined at the same time in many cases. The effect on fungi, eelworm, etc., was also determined roughly.

2. It was found that nearly all the substances disappeared from the soil fairly quickly and at the same time the numbers of bacteria fluctuated. The usual march of events was that the bacteria were reduced in number for the first few days, then rose to a maximum and finally fell slowly towards normal. The whole fall was sometimes very slow and the whole process was much slower in field soil than in the richer, lighter and better aerated greenhouse soils.

3. Aeration was found to have great influence on the rapidity of the changes.

4. The increase of the bacteria during the early days of an experiment varied in the same direction as the molecular weights and heats of combustion of the antiseptics and is attributed to the latter property. Naphthalene, for instance, which has a large heat of combustion, caused enormous rises, while benzene with its lower heat caused smaller rises.

5. This rise was independent of the effect of the substance on the protozoa. Both naphthalene and toluene in large doses cause high rises; the first has no effect on the protozoa, while the latter kills all amoebae and ciliates.

6. Similar results were obtained when the experiments were made on soils already free from protozoa, such as a field soil which had been in bottle for 76 years, soil in which they had been killed by steaming, and soil in which they had been killed by antiseptics. If the protozoa were killed by the use of a strong dose of a suitable antiseptic and the soil were then set aside for a long period, a second dose caused an even greater rise than the first.

7. It is therefore concluded that the rise in the number of the bacteria is largely due to the feeding effect of the antiseptic on the bacteria and not only to the destruction of the protozoa, and that the increased fertility observed by Russell and Hutchinson is to be attributed in large measure to the activity of the greater bacterial population in breaking down the organic matter of the soil. Bacterial rises following treatment with lime or steam are similarly caused in part by the preparation of the plant residues.

8. Aliphatic compounds cause quicker but smaller rises than those of aromatic series.

9. The introduction of a CH_3 group into the benzene ring lessens toxicity to soil organisms, while a single Cl or nitro-group increases both toxicity and stability in the soil.

APPENDIX.

It will be useful to arrange the various nitro and chlorine derivatives of benzene in descending order of toxicity to eelworm fungi and protozoa, so that the effect of introducing the various groups may be emphasised. When phenol, cresol and anilin were examined, similar effects were to some extent apparent.

Table XXXIV.

Chemical	Group introduced into ring	Protozoa		Eelworm		Fungi		Effect on bacteria
		Dose which cuts down	Fatal dose	Dose which cuts down	Fatal dose	Dose which cuts down	Fatal dose	
Benzene ...	—	$M/10$	—	$M/50$	$M/10$	$M/10$	—	High rises
Chlorbenzene	Cl	—	—	—	$M/50$	$M/10$	—	No rise
Dichlorbenzene	2Cl	—	—	—.	$M/200$	Strongly $M/10$ Strongly $M/50$	—	Poor rise
Nitrobenzene ...	NO_2	—	$M/10$	$M/200$	$M/100$	Very strongly $M/50$ to $M/10$	—	Rise
Dinitrobenzene	$(NO_2)_2$	$M/10$ (only ciliates left)	—	—	$M/200$	Very strongly $M/10$	—	High rises
o-Chlornitrobenzene ...	$ClNO_2$	Above M 50	—	—	$M/200$	$M/50$	$M/10$	No rise
Nitrodichlorbenzene ...	$NO_2.2Cl$	Above $M/50$	—	$M/200$	$M/100$	$M/50$	—	No rise
Chlordinitrobenzene ...	$Cl(NO_2)_2$	$M/500$	$M/200$	—	$M/200$	$M/500$	$M/200$	High rise
Dichlordinitrobenzene A ...	$2Cl(NO_2)_2$	—	—	—	$M/60$	Very strongly $M/60$	$M/30$	Fair rise
„ B ...	—	—	—	—	$M/100$	Very strongly $M/50$	$M/10$	

Table XXXV.

Lists arranged in descending order of toxicity to

Eelworm

1. Chlordinitrobenzene
   o-Chlornitrobenzene
   Dinitrobenzene
   Dichlorbenzene
2. Nitrodichlorbenzene
   Nitrobenzene
   Dichlordinitrobenzene
3. Chlorbenzene
4. Benzene

Fungi

1. Chlordinitrobenzene
2. Dichlordinitrobenzene
3. o-Chlornitrobenzene
4. Nitrobenzene
   Dinitrobenzene
5. Nitrodichlorbenzene
6. Chlorbenzene
   Dichlorbenzene
   Benzene

Protozoa (incomplete)

1. Chlordinitrobenzene
2. Nitrobenzene
3. Benzene
4. Dinitrobenzene

Stability in soil

1. Chlorbenzene
2. o-Chlornitrobenzene
   Nitrodichlorbenzene
   Dichlorbenzene
3. Dichlordinitrobenzene
4. Nitrobenzene
5. Chlordinitrobenzene
   Dinitrobenzene
6. Benzene

In the last list the substances are arranged in the order of their stability.

The above lists indicate that, generally speaking, benzene is least

toxic and chlordinitrobenzene most toxic to soil organisms, yet they stand next to one another as regards quick decomposition and provision of energy for bacterial growth. A chlorine group by itself, or with a nitro group, or again, two chlorine groups with one nitro group produce great stability in soil. One or two nitro groups or two nitro groups combined with one chlorine group form an unstable product on which soil bacteria thrive abundantly. Much quantitative work remains to be done before such a table can be reliable, *e.g.* weaker doses of the two forms of dichlordinitrobenzene might be found effective as pest killers, thus necessitating a change in tabulation. Yet as a preliminary qualitative basis for rigid quantitative work these lists are useful. Regarding the application of these reagents to infected soil, two points urgently require further investigation:

(*a*) Is the action on fungus spores and eelworm fatal, or merely inhibitive while the reagent is present? If it is inhibition, does the reagent injure the growing crop?

(*b*) Will the spores revive after the disappearance of the inhibitive reagent in cases where it serves as bacterial food?

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THE MANURIAL PROPERTIES OF LEAD NITRATE.

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INTRODUCTION.

THIS investigation was undertaken in response to an enquiry respecting the value of lead nitrate as a source of nitrogen for manurial purposes. The ordinary commercial outlets for lead nitrate at the present time are in the manufacture of paper, printing ink and paint, also, in calico printing, where it is employed as a mordant. In the early stages of the War it was used as a shell-filling compound. Scrap lead is generally employed for its manufacture; a process which is protected by several patents[1]. The trade in this commodity is relatively small.

Lead compounds like a number of other mineral salts are well known as plant and animal poisons. While this class of substance is poisonous at relatively high concentrations, at low concentrations many of them are plant stimulants. As stimulants they differ from the ordinary plant nutrients in that they are not indispensable and that plant growth is not controlled by their supply, nor do they appear to form a normal constituent of the tissues of plants. As to the mechanism of their action upon the vital processes in plants, a discussion upon this point is outside the object of this short paper.

The action of lead nitrate upon plant growth is complicated by the fact that (1) the salt supplies nitrogen in an available form, (2) its fertilising properties may be completely destroyed by the toxicity of the lead, and (3) interaction between the salt and the soil constituents. With the object of elucidating some of these points, water culture, pot and field experiments, were undertaken.

WATER CULTURE EXPERIMENTS.

Oats var. *Potato.*

These were carried out with the object of determining the toxic and stimulating limits of lead nitrate in solution. Stimulation was measured by the increase produced in the total weight of plant. In certain cases,

[1] Lead nitrate, manufacture of, W. Mills: Eng. Pat. 6143, 1904; U.S. Pat. 779,092, 1905.

the weight and length of root and stem were recorded separately. The death point was taken as being the stage when the plant flagged and turned yellow.

To eliminate as far as possible errors due to parental differences in individual grains, "singles" were separated from a number of inflorescences. The grains varied in weight from 0·04 to 0·045 gram. Germination was produced in sawdust and when the blade was 3″ long the complete plant was lifted and transferred to the solution under experiment. The average weight of plant at this stage was 0·129 gram. The plants were held in position by placing through a hole in a cork plugged with cotton wool, inserted into the neck of a bottle; the roots being immersed in the solution. Brown paper was wrapped round the bottle. Corks and other utensils were sterilised in boiling water. In the making of the distilled water a tin condensing worm was employed. It was not practicable to use a distilling apparatus wholly of glass and a silver or platinum apparatus was not available.

The average result of seven separate experiments is given below.

| | Average weight of plant in grammes | |
Concentration	Lead nitrate	Lead chloride
1 : 78	0·128	—
1 : 156	0·143	0·130 saturated solution
1 : 312	0·150	0·141 ,,
1 : 625	0·159	0·147 ,,
1 : 1,250	0·195	0·162 ,,
1 : 2,500	0·199	0·171 ,,
1 : 5,000	0·201	0·196 ,,
1 : 10,000	0·215	0·196 ,,
1 : 20,000	0·230	0·222 ,,
1 : 40,000	0·279	0·224 ,,
1 : 80,000	0·299	0·231 ,,
1 : 160,000	0·282	0·242 ,,
1 : 320,000	0·280	0·244 ,,
1 : 640,000	0·274	0·277 ,,
Control	0·261	0·251 ,,

The figures are represented graphically in Fig. 1, p. 60.

Taking the control as representative of normal growth, it is evident from the curve that concentrations of the nitrate from about 1 : 40,000 to 1 : 640,000 caused stimulation, the maximum effect being produced at a concentration of about 1 : 80,000. Below 1 : 40,000 retardation occurred progressively with increase in concentration. Death occurred within eight days in all concentrations up to 1 : 20,000. With the chloride no stimulation occurred until a dilution of about 1 : 340,000 was reached. Toxicity was more pronounced with the chloride than with the nitrate.

SOIL CULTURE EXPERIMENTS.

A preliminary experiment was first carried out with oats and Italian rye grass, with the object of comparing the effect of nitrate of lead and of nitrate of sodium containing equal weights of nitrogen. The same number of seeds was sown in pots each holding 40 lbs. of soil (Holmes farm) to which was added a manurial dressing of superphosphate of lime and muriate of potash. A solution of nitrate of sodium containing 9·96 grams dissolved in 500 c.c.s of water and a solution containing the equivalent in lead nitrate was added to the respective pots in quantities of 125 c.c.s at a time. The control pots received an equal volume of water only.
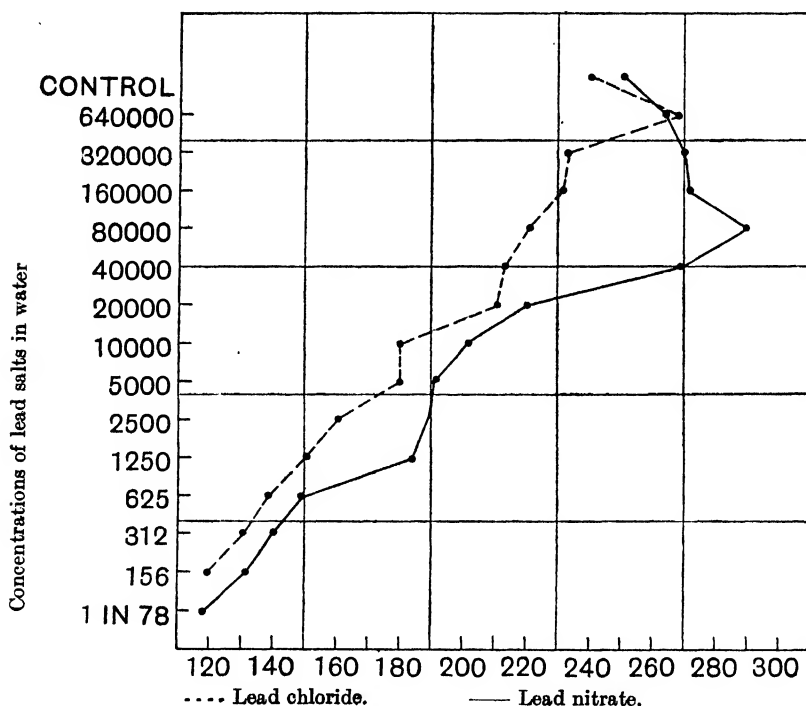
Fig. 1. Average weight of plant in grams.

Growth in the pots receiving nitrates was much stronger and the foliage was greener than in the control. Where lead nitrate was added it was noticed the plants possessed slightly broader leaf blades, and the colour of the foliage was, if anything, of a darker shade, compared with the plants to which nitrate of sodium was applied. The lead nitrate solution was three times the concentration of the strongest solution used

in the water culture experiments. The ratio of salt to soil was 1 : 1125. The result showed that the toxicity of highly concentrated solutions of lead salts such as the nitrate and the chloride is destroyed by the presence of soil.

As a result of the preliminary trial two series of pot experiments were carried out. Light sandy soil from Gargeston was used in series I and soil from Holmes farm in series II.

Series I consisted of three pots each in triplicate as follows:

Pot 1. Untreated.
  ,, 2. Nitrate of sodium.
  ,, 3.   ,,   lead.

The pots were sown with rape, a quick-growing plant. The weight of nitrate employed was the same as that used in the preliminary trial. One-half was sown at seeding and the remainder applied as a top dressing. The effect on the plant of the lead salt was again visible in the darker colour of the leaf and a crop was produced which appeared to be equal in growth to that produced by nitrate of sodium. Owing to damage caused by a storm of wind and rain the plants were not weighed.

Series II consisted of five pots in duplicate. Each pot was sown with oats (var. potato) and dressed with superphosphate of lime and chloride of potassium. The nitrate was applied in equivalent quantities, likewise the lead. The same number of plants were grown in each pot. Date of sowing May 30th, and of cutting September 14th, 1921.

The scheme of manuring and the weight of total crop (dry) are given below:

Pot 1. No nitrogen	250·0 gms.	
,, 2. Nitrate of sodium	309·1	,,
,, 3. ,, lead	293·7	,,
,, 4. Acetate of ,,	245·1	,,
,, 5. ,, ,, + nitrate of sodium	312·0	,,

By an inspection of the above figures it is evident that lead nitrate can be used with advantage as a substitute for nitrogenous manures such as nitrate of sodium. Acetate of lead used alone in the quantity employed had a slight toxic effect.

Lead poisoning of both crops and stock are known to occur in the neighbourhood of lead mines, the toxic compound generally being galena.

Griffith[1] found that the addition of galena to a fertile soil in quantities

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[1] Griffith, J. J. Influence of mines upon land and live-stock in Cardiganshire. *Journ. Agric. Sci.* **9**, Pt 4, p. 388, 1919. See also Plumbism, or lead-poisoning, by E. Morgan, *Journ. Univ. College of N. Wales Agric. Dept.* **8**, 29–41, 1915.

amounting to 0·4 per cent. of the total weight of soil only lowered the fertility to a comparatively slight extent.

The toxic and stimulating limits of lead nitrate in presence of soil was next determined. For this purpose further pot experiments with oats were carried out in the autumn of 1921. Two series were started in the month of October, the pots being kept under glass. Gargeston soil was used in each case.

Series I. Each pot received, in addition to nitrate, a dressing of superphosphate of lime and of muriate of potash.

Series II differed from the former in that chloride in place of nitrate of lead was used; equal weights of lead being applied. In other respects the pots (2 to 5) received the same dressing as No. 1. The salts were mixed with the soil before sowing the seed.

The treatment and average results obtained are shown in Table I below. Duration of experiment 73 days.

Table I.

Pot	Lead nitrate added Percentage of soil	Series I Lead nitrate Weight of plant (gm.)		Series II Lead chloride Weight of plant (gm.)	
		Total	Stem	Total	Stem
1	Sodium nitrate 0·044	2·800	2·164	2·897	2·452
2	{ „ „ · 0·022 { Lead „ 0·045	2·953	2·245	3·394	2·562
3	Lead nitrate 0·09	2·495	1·836	2·941	2·190
4	„ „ 0·17	2·512	1·730	2·817	1·874
5	„ „ 0·34	2·280	1·612	1·464	0·762

The following conclusions are drawn from the above figures:

1. Lead nitrate is slightly superior as a nitrogen fertiliser to that of nitrate of sodium; the superiority being due to the stimulating action of the lead.

2. Applied in amounts equal to 0·09 per cent. of the weight of soil lead nitrate is slightly toxic. At a concentration of 0·34 per cent. serious harm to the crop was visible.

Voelcker[1] found that the addition of lead salts up to 0·1 per cent. of the weight of soil caused no decrease in the total yield of crop.

FIELD EXPERIMENTS.

Small plots of one two-hundredth of an acre were laid out at Holmes Farm in the spring of 1921 and sown with oats var. potato. The scheme of manuring is shown below. Nitrate of sodium was applied at the rate of

[1] Voelcker, J. A. Pot culture experiments. *Woburn Expt. Station*, p. 30, 1915.

1 cwt. per acre and the lead salts containing the equivalent nitrogen. The salts were finely ground and mixed with sand so as to ensure uniform distribution on the soil. The seed was sown on April 28th. One-half the nitrate was applied on May 24th and the remainder on June 5th.

Plan.

| No manure | Lead nitrate | Sodium nitrate |
| Sodium nitrate | No manure | Lead nitrate |

The plants growing on the lead nitrate plots again showed slightly greater leaf development and were a shade darker green in colour compared with the plants on the nitrate of sodium plots. The average size of grain and the date of ripening were practically alike for the manured plots. Owing to losses of grain from attacks of birds it was of no advantage to weigh the produce, but samples of both grain and straw were kept to test for the presence of lead. These plots are to remain down for a number of years, the treatment being the same each year; the produce to be weighed and analysed.

No trace of lead could be found in the produce from the plots treated with the lead salt. Griffith found that oats growing on soil contaminated with lead (0·41 per cent.) yielded an ash containing 0·07 per cent. of lead. The concentration of lead in the present case, however, would only be about 1 in 10,000.

REACTION BETWEEN SOIL AND SOLUBLE LEAD SALTS.

It is clear as a result of the foregoing experiments that soil reduces the toxicity of lead salts in solution. The reduction is so pronounced that a concentration in water poisonous to plants, produced in presence of soil a marked stimulation. The cause of the reduction in toxicity would appear to be due to the removal of the greater part of the lead from the sphere of action. Retention in a more or less insoluble condition of the basic radicle of many soluble salts is one of the characteristic features of soil properties. Interchange of bases between soil constituents and salt or adsorption by colloid complexes in soil largely account for the phenomena of retention. In the present case insoluble sulphate and carbonate of lead would probably be formed also. Jensen[1] found that quartz alone reduced the toxic effect of poisonous salts particularly in dilute solution.

[1] Jensen, G *Botanical Gazette*, **43**, p. 44, 1907.

In 1921 some experiments with soil and aqueous solutions of lead salts were started, an account of which is given below.

100 grams of soil were shaken up with a solution of lead nitrate 1 : 10,000 and after standing with occasional shaking for 24 hours, the liquid was poured off, filtered and tested for lead. No lead could be found.

In another case soil to a depth of $8\frac{1}{2}$ inches was placed in two inverted Winchester bottles with the bottom cut off. In No. 1 water and in No. 2 the strongest nitrate solution used in the experiment on p. 59 was drained through and the drainage tested for lead. Reactions for nitrates were obtained in both cases but no trace of lead by colorimetric tests could be found, except in No. 2 and then only after successive quantities of the lead solution had been filtered through.

Retention of lead varies according to the ratio of the reacting substances, as shown in the results of the following experiment. The same initial concentration and volume of lead salts was used, but increasing quantities of soil. Oat plants were grown in the liquid after it had remained in contact with soil for 24 hours, poured off and filtered. The plants were weighed and the average figure is given below. In the control water and soil were used. The experiment started on April 5th and was completed by April 26th, 1922.

No.	Soil : Pb (NO$_3$)$_2$	Av. weight of complete plants (gms.)
1	1 : 0·064	0·304
2	1 : 0·032	0·422
3	1 : 0·016	0·633
4	1 : 0·008	0·943
5	1 : 0·004	1·094
6	Control	1·337

Solutions Nos. 1 to 3 were poisonous. No. 4 slightly toxic. No. 5 plants quite healthy.

Colorimetric tests for lead showed that the amount left in solution decreased as the proportion of soil increased. No. 5 still gave a test for lead.

The soil left in No. 1 Winchester after standing for some time had a rather unpleasant odour, whilst the soil left in No. 2 had not. To account for this it was thought that the presence of the lead might have affected the bacterial flora of the soil. With the object of obtaining an indication of this the rate of production of nitrates in the soil was determined.

LEAD SALT AND NITRATE PRODUCTION IN SOIL.

Three large Buckner funnels each holding 7 lbs. of soil were fixed into a box, sunk into the soil and left exposed several months in the summer of 1921. No. 1 had sodium nitrate, No. 2 lead nitrate in the same proportion as used in pot experiment (p. 60) mixed with the soil, No. 3 soil alone. The drainage water was collected, evaporated and tested for nitrate by colorimetric tests. The tests showed definitely that the drainage water from the soil to which was added lead nitrate contained more nitrate than the drainage water from the soil to which nitrate of sodium was applied. An aliquot portion was evaporated to dryness, ignited and weighed; the weights being:

No.	Total solids (gms.)	Ash (gms.)
1	0·847	0·492
2	1·000	0·492
3	0·51	0·327

Further tests on the above lines are being carried out.

CONCLUSIONS.

Lead nitrate, as a source of nitrogen for fertilising purposes, is equal to nitrate of sodium when applied in quantities equivalent to those employed in agricultural practice. Its effect on the plant was to produce a slightly broader leaf blade and a deeper shade of green compared with the effect produced by nitrate of sodium. No difference in root development was observed.

Used in the amounts referred to, no trace of lead could be found in the plant, neither could any lead be detected in a solution made by extracting the treated soil with water.

Toxic and stimulating limits of lead nitrate and lead chloride were determined in water and in soil cultures. Except in solutions of fairly high concentration soil adsorbs the lead and destroys the toxicity of soluble lead salts.

There was evidence to show that the addition of lead salts increased the rate of nitrification in soil.

(*Received May 8th*, 1923.)

INVESTIGATIONS ON YIELD IN THE CEREALS[1]. I.

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CONTENTS.

PART II (*continued*).

§ VII. TILLERING.

Considerations which are dealt with at length in Appendix III governed the form of the investigation upon tillering. In essence, the intention was to compare the pure lines *P* and *A* and to explore the possibilities of making accurate determinations of tillering. For reasons discussed in the Appendix, special steps were taken to secure for observation a population of plants which had had as great a uniformity of conditions as was attainable. The selection and sowing of the seed have been described in § I above. To promote further uniformity, plants of the following categories were excluded from observation: (i) all the plants of end rows; (ii) the end plants of all rows; (iii) plants which did not germinate on 26. iii. or 27. iii. (these days constituted the "modal" 2-day

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period of germination for the whole population); (iv) plants damaged to a noticeable degree by wire-worm or any other such agency; (v) plants whose immediate neighbour(s) in the row had died.

There was a separate record for every plant and on four occasions in the first two months of growth the number of tillers on every plant was counted. Later counts proved impossible because of the size of the plants. On the first occasion the number of leaves also was recorded.

Emphasis must again be placed on the fact that every tiller large enough to be seen, was counted. It was impossible to record the sizes of tillers and equality in number often accompanied disparity in size. Thus of two plants, each of two tillers, the first might have two small tillers and the other, one large and one small.

The results of the counts are contained in Tables I–XI and the key which precedes these tables indicates the date to which every table refers, the numbers of the beds, and the spacing intervals. Every bed consisted of two plots, the one containing P the other A, and the varieties alternating from left to right of the key. Throughout the tables the errors affixed to the means are standard errors ($\sigma/\sqrt{n}$).

An idea of the general stage of growth reached at every count is afforded by § III above—Field Notes on Growth.

The results of the counts may now be examined in turn:

(A) *Leaf Development on* 21. iv.

After 21. iv. the leaves were too numerous for reliable counting and no other leaf counts were made. Up to that date the leaves of the tillers had not unrolled so that number of leaves refers to main-axis leaves only. Tables I A–IV A contain the results.

The eight distributions (for two pure lines at two spacings and in duplicate beds) are all clearly bi-modal. All have their first mode at 3·0 (3 leaves and no tillers). The position of the second mode is not quite constant. For the 2-inch spacing it is in all cases at 4·1; for A at 4-inch spacing it is also in this position; but for P at 4-inch spacing it is at 4·2. In all cases duplicate sowings, *e.g.* P in Bed 5 and Bed 6 have identical modal positions. Thus the bi-modality of the distributions is well marked and concordant. It will be observed that in every distribution some 90 per cent. of the population is concentrated at the modes (3·0 and 4·1–4·2). These two classes seem, indeed, to mark definite stages of morphological development, the protrusion of the first side tiller being nearly always synchronous with the appearance of the fourth leaf of the main axis. Harlan[11] has shown that a number of well-marked morpho-

logical stages can be demonstrated in the early development of many barley varieties.

For a comparison of the tillering of *P* and *A* and of growth at the two spacings it is convenient to tabulate the percentages of plants at or beyond the 4·1 stage on 21. iv. The results are:

	4-inch		2-inch	
	Bed 5	Bed 6	Bed 7	Bed 8
P ...	72·66	84·47	44·49	60·95
A ...	70·05	76·00	63·07	62·51

It is seen that at 2-inch spacing *A* is in advance of *P* in both the beds. The order is reversed in both the 4-inch beds. No definite reason can be advanced for this reversal but in the light of the inter-varietal root difference described in § III above an explanation might, perhaps, be hazarded.

The plants have already—three weeks after germination—given evidence of the influence of spacing upon tillering and leaf formation. That inter-plant competition may be demonstrated at an even earlier stage is apparent from the results recorded by Prescott[29] for maize.

Although the distributions of Tables I A–IV A are very regular, it is significant that they furnish clear evidence of the beginnings of great inter-plant developmental differences. Thus in Table III A, for instance, 38 per cent. of the plants are at 3·0 while 31 per cent. at 4·1, a morphological "step" in advance. Such early differences are likely to be pre-determinative and do not augur well for the uniformity of the population at maturity. They illustrate in a forcible way the need for some system of repeated selection of samples such as is suggested in § VIII.

In the data which has been given the actual numerical differences between *P* and *A* are too small to signify. Possibly, however, the reversal of their order of tillering from the 2-inch to the 4-inch spacing does reflect a varietal difference.

(B) *Tillering on* 21. iv.

Tables I–IV contain the data.

Comparison of *P* and *A* in terms of the mean number of tillers per plant reveals, perhaps necessarily so, a difference between the 2-inch and 4-inch spacings of the kind recorded in regard to the proportions of plants which had reached or passed the 4·1 stage. *A* is ahead of *P* for the 2-inch spacing and behind it for the 4-inch. The actual differences are, however, not significant in terms of the standard errors (bottom row of table).

The sequence of the means across the plots is to be noticed. Comparing

members of duplicate pairs, *e.g. P* Table IV and *P* Table III it is seen
that there is always a decrease from right to left (except in *A* Tables IV
and III). This seems to indicate a progressive falling away of soil fertility
or some other circumstance affecting growth.

For an erratic attribute such as "tillering" the standard errors of the
means are not very great and to this extent the method of the experiment
appears satisfactory.

(C) *Tillering on* 28. iv.

The observations are for Bed 8 (2-inch spacing) only and are con-
tained in Table V. The distributions are wider than those of the previous
count but the standard errors of the means remain of reasonable magni-
tude. The difference of the means is again in favour of *A* but it is small
in comparison with the errors involved.

(D) *Tillering on* 7. v.

One bed for each spacing was counted, viz. Bed 5 for 4-inch (see Table VI)
and Bed 8 for 2-inch (Table VII). The distributions are again wider, the
significant ranges being 2–6 tillers per plant for 2-inch spacing and
2–7 for 4-inch. Standard errors of means continue to be of practicable
magnitude and the distributions are free from irregularities. It is un-
necessary to discuss the *P–A* numerical differences since they are again
too small to signify.

(E) *Tillering on* 25. v.

This, the last count before harvest, is for plants two months after
germination. The magnitude of the errors of observation due to sampling
at this stage may be judged from Table VIII. Here the errors are bigger
than in any other case but a difference of unity between two means
would be significant. The criterion is the standard error—a higher one
than that of probable error which is customarily employed.

Again, no significant difference between *P* and *A* is traceable but
the gradation of soil character suggested by the results of 21. iv. is once
more represented in the sequence of corresponding means from right to
left across the beds.

(F) *Conclusions upon P and A.*

Table XII, a summary of mean values for the successive counts,
shows that:

In Bed 8 *A* first exceeds *P*, is then equal to, and finally below it.
     ,,   7 *A* exceeds *P* consistently.
     ,,   6 *P* exceeds *A* consistently.
     ,,   5 *P* exceeds *A*, is then below it, and finally again exceeds it.

Thus the week to week sequence is irregular. And further, as has been pointed out, the numerical differences between the two varieties are in all cases not of significant magnitude in terms of the errors involved. It is to be concluded therefore, that in the circumstances of the experiment, *P* and *A* show no positive difference in tillering up to and including 25. v. Possibly an exception is to be found in the reversal of order of tillering between the 2-inch and 4-inch spacings [see (A) and (B) above]. Even in this respect the difference must be very small and could only be established by further test.

Ear-formation is dealt with in subsequent paragraphs but it may be observed from Table XIII that *P* and *A* show, in regard to that attribute, no positive difference.

General field experience suggests that in most years *A* tillers more freely than *P*. It is possible that the severe drought of 1921 set a sharp limit to tillering in both the varieties and so masked an inherent potential difference between them. From one year's observations such a possibility cannot be tested.

(G) *Conclusions concerning the Method Employed.*

In comparison with other methods, a considerably increased accuracy was achieved. It resulted from careful seed selection, regular sowing, and the exclusion of all plants which had obviously been exceptionally circumstanced. Such exclusion greatly reduced the experimental population. A severe wire-worm attack was largely responsible for the losses and, it must be remembered, the death of one plant involved the rejection of two others. For Bed 7 the losses are represented by the following table:

Variety	Sown	Excluded because of end position	Theoretical balance	Actual balance on 21. iv.
P...	598	94	504	389
A...	598	94	504	443

The counting of tillers on young plants is very laborious and every step which, by promoting uniformity, can reduce the permissible size of the experimental population, is important. A procedure such as that described promises to be practicable in point both of accuracy and of manipulation.

§ VIII. Time of Flowering.

For reasons fully given in Appendix V it has been concluded that:

(i) Time of Flowering is a reliable varietal "character."

(ii) The relation of the flowering times of the individual tillers of the plant may be connected with the yielding power of the variety.

In hybridising many of the barleys (including both P and A), experience has shown that the emasculation of the female parent should be performed when the awns of the ear have emerged about one inch from the top leaf sheath. At this time the anthers are sufficiently green to make their removal safe and the stigma has just become receptive. Consequently this degree of awn emergence was taken to indicate "flowering time." Harlan (see Appendix V) has already recorded the successful use of such an index.

Red wool was tied to the first ear of every plant, white, blue, and green being used in turn for the subsequent ones. The date of "flowering" was recorded for every ear of every plant and thus it was possible to identify the first, second, etc., ears and to obtain their flowering dates.

As for observations of tillering (see § VII above), data were derived solely from plants which had satisfied certain specifications as to conditions of growth. In the tables of results and in the text "emergence of the ear" implies the emergence of the awns from the top leaf sheath to a distance of an inch and this, as has been explained, is taken as indicative of "flowering." Observations were made on the mornings of alternate days commencing on 11. vi., the day on which ears began to emerge. They ceased on 19. vi. for after that date the few plants that pushed out more ears were almost entirely ones which did not meet the requirements of the specified uniform conditions. Beds 5 and 8 had been seriously depleted by flowering time for dry-weight determinations and no data were derived from them.

In discussing the data, the following subjects are considered, but their inter-relations do not permit of rigidly paragraphic treatment:

(i) Comparison of P and A.

(ii) The inter-relations of the tillers of the plant.

(iii) The relation of time of flowering to tillering in the earlier stages.

(iv) The relation of time of ripening to the number of ears ripened per plant.

Table XXIII shows the date of emergence of T_0 for the complete populations. For the 2-inch spacing A is somewhat earlier than P and for the 4-inch the difference is quite pronounced. In this comparison, as in those that follow, it is not desirable to compare mean values, for the differences involved are small, and the counts were made on alternate days. General form of distribution, position and prominence of its mode etc., are, in the circumstances, the safest criteria. It seems that at both spacings A is a little earlier than P. The fact that, for the greater spacing, A is earlier than for the lesser, is not certainly significant—the difference

is but slight. For both varieties the dispersion of the distribution is less at 2-inch spacing than at 4-inch but even the greater dispersion is sufficiently limited to justify the conclusion that the date of emergence of T_0 may be accepted as a "constant character."

The number of plants at the 4-inch spacing is too small to allow further analysis and the remaining considerations therefore relate exclusively to the plants grown at 2-inch spacing. For these (*vide* Table XXIII) the modal day of ear emergence was 13. vi. for both P and A. On the assumption that the date of emergence of T_0 is an index of development, those plants whose T_0 emerged on 13. vi. may be regarded as a more uniform population than the whole bulk and therefore as a suitable one for determining the time relationship of T_1 and T_2. Table XXIV contains the required data. P is slightly in advance of A in the emergence of both T_1 and T_2—the difference is very small—and for both varieties the mode of the T_1 distribution is about mid-way between 13. vi. (the T_0 date) and 15. vi. while the T_2 modes are at 17. vi. Thus in both the varieties the ears of T_1 and T_2 emerged very soon after that of T_0. The right-hand column of the Table shows that over 90 per cent. of the plants put forth their T_1 in the period 11. vi.–19. vi. while 70 per cent. of them put forth, in addition, their T_2. It may be noted that for plants whose T_0 emerged on 15. vi. the T_1 and T_2 distributions have well-marked modes at 17. vi. and 17. vi.–19. vi. respectively (the distributions are similar to those of Table XXIV). Thus it seems that date of emergence of T_0 is correlated with date of emergence of T_1 and T_2. To evaluate actual correlation coefficients a very great number of observations would be required. The fact that the method used is able to demonstrate the small time difference for T_1 and T_2 between the plants which put forth T_0 on 13. vi. and 15. vi. respectively indicate the sensitiveness of the correlation.

It will be recalled (see Table III A) that the distribution of number of "leaves and tillers" on 21. iv. was bi-modal—the modes were at 3·0 and 4·1—and it was suggested that these values should be regarded as denoting successive stages of morphological development. In Table XXV are given the frequency distributions of the date of emergence of T_0 for the plants which constituted the "modal classes" on 21. iv. For both the classes there is a close similarity between P and A and for both the varieties it is clear that the (4·1) population was ahead of the (3·0) in the emergence of T_0. The difference is small but it is to be regarded as significant—the time of flowering of T_0 has already been shown to possess a high constancy. There is evidence, then, to the effect that early

leaf formation and tillering are correlated with subsequent behaviour: or in phraseology sometimes employed, that early conditions have a marked "pre-determining" effect on after development.

The remaining tables afford further test of this principle. They indicate, briefly speaking, the following facts:

Table XXVI—the population which was (4·1) on 21. iv. put forth more ears per plant during the period 11. vi.–19. vi. than did the population which was (3·0) on 21. iv.

Table XXVIII (compare with Table III A)—the plants which put forth T_0 on 13. vi. (and thus were in advance of the general population on that date) were, on 21. iv., similarly in advance in leaf and tiller formation.

Table XXIX (compare with Table X)—the plants that put forth T_0 on 13. vi. were similarly in advance of the general population in tillering on 25. v.

Table XXVII (compare with Table XXIII) shows that, in the emergence of T_0, plants which succeeded in ripening 3 or more ears at harvest were in advance of the general population. (See Table XIII for percentages of the whole population which ripened < 3, 3, and > 3 ears.)

It is to be observed that the advancement displayed in Tables XXVI–XXIX is upon the whole population. The advance upon the *rest* of the population is, of course, more marked. In Table XXIX the average number of tillers per plant is $P = 3\cdot34$; $A = 3\cdot51$ while in Table X (general population and therefore including the plants of Table XXIX) it is $P = 3\cdot16$; $A = 3\cdot33$.

It is concluded that, for the populations concerned, P and A showed a very close similarity in time of flowering whether judged by the time of T_0 for the plant or by the inter-relations of T_0–T_1–T_2. The possible relation between the time data for T_1 and T_2 and yield, is reserved to a later paragraph: but it is quite clear that T_1 and T_2, arising much later than T_0, hasten to the flowering stage and reach it only 2 and 4 days (respectively) later than T_0. This fact is of some interest as the following hypothetical case shows. Consider a plant which was 4·1 (4 leaves and 1 tiller) on 21. iv. Its main axis (T_0) emerged from the ground on 27. iii. (only such plants were included in the observations). Its second side tiller (T_2) cannot have emerged from the leaf sheath before 22. iv. at the earliest. If the emergence of T_0 from the soil and of T_2 from the leaf sheath are roughly comparable (the sizes of the two young shoots are roughly equal) then the minimum difference in age of T_0 and T_2 at flowering time must be the period 27. iii. to 22. iv., *i.e.*, 26 days.

Nevertheless, in an average case, only 2–4 days separates the times of flowering. The shorter period of development is correlated with a smaller yield of grain (*vide* §§ IX and XI which follow) and the ages of the several tillers are likely to be related to the yield of the whole plant. It is quite clear that the origin and nature of inter-tiller differences of final development must be sought in the juvenile development and not in the times of flowering of the tillers, for these are levelled up in a manner which obscures the earlier differences. The data afford no evidence concerning T_3, etc., for very few plants ripened more than three ears. It was pointed out in § III that many late tillers died down at an early age.

Corresponding to the rapid advance towards flowering of T_1 and T_2—which results in their flowering very soon after T_0—there is evidence of similar rapidity on the part of the main axes of plants which, early on, were poorly developed. For example (see Table XXV) the (3·0) plants (on 21. iv.) though morphologically well behind the (4·1) (on 21. iv.) flowered (T_0) only some 2–4 days later. The tendency of plants and tillers variously developed in the early stages, to flower all within a limited period, is indeed, very well marked.

Some interest attaches to the suggested ordered relationship between development in successive morphological phases of the life of the plant. Great differences in an early stage are to be expected to be followed by differences in all subsequent stages but the results here described indicate that the phenomenon is demonstrable even when the juvenile differences are small. From the general consistency of the results it is concluded that the morphological features observed—numbers of leaves and tillers, dates of emergence of T_0, T_1, etc.—are useful guides to development. It is explained in a later paragraph that in efforts to determine rate of increase of dry matter, etc., the difficulty of drawing uniform and representative samples was almost insuperable. The results above described suggest an improved method of sampling. Single plant records, the exclusion of outside and other objectionable plants, and periodic counts of tillers, etc., would have to be made (as described above). Starting from the observation of date of germination, the "modal-class" plants of one week would, alone, be observed in the following week (the other plants would, of course, be left to grow on). From the distribution (of number of leaves or whatever character was most appropriate at the time) the "modal class" would again be determined and this alone would be observed at the next observation time following. Every week, the dry weight or other experimental sample would be drawn from the "modal class" of the week. Large sowings would be necessary for this

method but it appears to afford a measure of improvement upon the sampling methods commonly practised and which, for barley, have been found most unsatisfactory (see § X).

§ IX. Weight of Grain, Weight of Straw, their Ratio, and Number of Grains for the Individual Tiller.

Reasons have been advanced in Part I for the study of "yield per plant" as a necessary step towards the full understanding of "yield per acre." Reliable "per plant" determinations are obtainable by averaging a suitable number of observations upon single plants. The number of single plants required, *i.e.* the size of the sample, is an important consideration. For a specified degree of accuracy, it depends upon the magnitude of plant fluctuation in respect of the attributes concerned. The greater this fluctuation, the larger the "sample" which must be observed, and therefore the greater the labour. In plant breeding, the reduction of routine labour is of first importance so that it is very desirable to investigate the phenomena which regulate its amount— plant to plant fluctuations. To some extent, such fluctuation may be lessened by the special precautions in growing and sampling material, which have been described in connection with "tillering" (§ VII) and "time of ripening" (§ VIII). Precautions involve extra labour and thereby to some extent counterbalance the saving which results from reduced fluctuation. It is necessary then, to assess the merits and demerits of such precautions and to test the possibility of their leading to reliable "per plant" determinations from samples of the size, say, of an F_3 family.

A direct study of the nature and amount of "plant to plant fluctuation" is therefore necessary. Experience with graminaceous plants indicates that the most obvious and potent factor in this fluctuation is likely to be the phenomenon of "tillering." Plants which tiller have a distinctive constitution. There is a seminal root system whose precise share in the activities of the whole plant has never been determined, and in addition, every tiller develops an adventitious root system of its own. Whether, among the tillers, there can occur a translocation and interchange of the products of root absorption and photosynthesis, it is impossible to say. Possibly a graminaceous plant should be regarded not as a unit—in the sense that a plant of *e.g. Helianthus annuus* is so regarded—but as a colony of individuals the younger at first dependent on the others but later, self established and competing with them.

In the absence of knowledge upon these fundamental points, it is difficult even to surmise as to the probable relations subsisting between

tillers. That the tillers tend to display a gradation in size is a very familiar fact but to formulate a working theory of yielding power, it is necessary to know the form of this gradation and the extent of its constancy.

With the purpose of casting some light upon this matter, tiller by tiller weighings of grain and straw were made for selected P and A populations. To make the test a crucial one, these populations were rigidly selected on lines which appeared likely to ensure a high degree of uniformity. The requirements imposed in drawing the sample have in part been described already but that the significance of the method and the results may be properly appreciated, the whole must be completely stated here. The plants were drawn from Bed 7 (2-inch spacing) and no plant was included unless it:

(i) Germinated on 26. iii. or 27. iii.—the days on which the great majority of the P and A populations germinated (the modal days).

(ii) Appeared to have remained undamaged by wire-worm, accident, or in any other manner throughout growth.

(iii) Was a non-terminal plant of a row or come from a non-end row of the Bed.

(iv) Had a surviving plant on each side of it throughout life.

(v) Put forth T_0 on 13. vi.

(vi) Put forth T_0, T_1 and T_2 within the period 13. vi.–19. vi. (both inclusive).

(vii) Ripened 3 and only 3 ears at harvest (this was the modal number for the complete P and A populations).

(viii) Not damaged during harvesting or storage. [Dried leaves are apt to be lost, grains to fall, etc., from ripe plants and there were heavy losses from this cause.]

In the preparation of the ground, the selection and sowing of the seed, etc., great care was exercised.

The weights recorded are for air-dry and not oven-dry material. At harvest, every plant was enclosed in a stout paper wrapping and stored in the laboratory. Under such conditions changes in water content are small. Check-weighings of straw and grain samples thus stored were made daily over a three month period and the changes were less than 1 per cent. For every tiller the following determinations were made:

$$G = \text{weight of grains.}$$
$$S = \quad\text{,,}\quad \text{straw.}$$
$$R = \quad\text{,,}\quad \text{rachis.}$$
$$n = \text{number of grains.}$$

It was impossible to prevent small pieces of lamina of the bottom leaves, etc., from becoming detached in the packet and impossible to tell from which tiller they came but such losses were too small to upset the results at all seriously.

Only 24 plants of P and 31 of A from Bed 7 fulfilled all the requirements of uniformity and these alone were weighed tiller by tiller. Printing costs make it impossible to publish the complete weighings and only so much of the collected data will be given as is absolutely essential. The numbers of observations are, of course, inadequate to strict numerical deductions—correlations, etc.—but they suffice for certain qualitative inferences, as to inter-tiller relationship and serve to illustrate the fluctuation.

In the values of G, S, n, and G/n (average weight of a single grain) there is observable, from the full data, a clear tendency towards the gradation T_0-T_1-T_2, to which reference was made above. But, although the plant populations were so rigorously selected, even the sequence $(T_0 > T_1 > T_2)$ is not displayed by every plant in respect of these four attributes. The ratio G/R—grain weight to rachis weight—is extremely irregular showing neither constancy from plant to plant nor regularity of sequence among the tillers of the individual plants. For $G/(S+R)$—ratio of grain to straw—the constancy and regularity are more marked than for G/R but, even so, the order of magnitude is T_0-T_1-T_2 for some plants, T_1-T_0-T_2, for others, and, indeed, all the possible orders are exemplified.

Clearly then, the plant populations here considered, despite precaution, are far from uniform. The dates of emergence of T_1 and T_2, given in Table XXX are fairly even and, of course, the date for T_0 was in all cases 13. vi. But in regard to yield of grain per plant the irregularity is very great as Table XXXI shows. In the table, the plants are simply cast into groups of close-lying individuals—there are no regular class limits. It will be observed that there is a very compact group of A plants varying in yield from 436 to 442 and perhaps the briefest and most forcible way of numerically illustrating the fluctuation above described will be to give the full data for this compact group. Table XXXII has been constructed for this purpose. It needs little comment. The proportions of the plant's yield of grain located in the separate tillers are decidedly fluctuable—compare, for example, columns 1 and 2 of the table. Straw-distribution is even more irregular and—*vide* column 5— two tillers may bear about the same proportion of the total grain and yet have very different weights of straw. The values of $G/S + R$ sum-

marise in a striking way, the lack of regularity. Table XXXIII presents the fluctuations for the whole P population from a rather different aspect. It shows that the value of G for T_0 is not necessarily—not even probably—an index to its value for T_1 or T_2 or for the whole plant. The average weight of a single grain is, perhaps, the most consistent of the "weight attributes."

The data of this paragraph do not contribute to a comparative study of P and A.

In regard to the practice of plant breeding from the standpoint of yield, these conclusions have a considerable interest. By taking precautions in growing and sampling, there has resulted a uniformity whose effect is valuable and apparent in work upon tillering and time of flowering. But the same precautions, reinforced by other and more drastic ones, fail to produce any well-marked uniformity in the weight attributes of single plants. It cannot be said that no increase in uniformity results from the precautions—experience with less carefully drawn samples vouches for this—but it has to be concluded that, full precautions notwithstanding, the chance of reliably determining yield per plant from small samples is a remote one. Judging by this investigation, for every 100 seeds planted, only about six finally acceptable plants will be obtained and these will be far from uniform. In plant-breeding practice, an F_3 family averages probably 150–250 plants. The seeds from which they are grown are likely to be much less uniform in size and maturity than those picked for this experiment and thus the proportion of "acceptable" plants might be less than 6/100. To these considerations must be added the fact that the complete F_3 families from a cross—usually 400–600 in number—would cover a much greater area than did the P and A populations and thus, almost inevitably, would involve considerable soil differences. All the difficulties which thus promise to confront attempts to compare F_3 families for yield per plant, lie in wait for endeavours to construct a theory of yielding power based on single plant performance; and in this case their effect is likely to be even more mischievous.

Fluctuation, as a universal phenomenon, is a subject of much interest; in a limited population of barley plants it has, perhaps, a special interest, for a certain amount of data is to hand concerning its possible causes. Thus, for the restricted P and A populations dealt with in this paragraph, it is known that so far as concerns germination, spacing, freedom from mechanical injury, time of emergence of T_0, and number of ears ripened at harvest, there was uniformity. For the seven plants of Table XXXII moreover, there was uniformity of total weight of grain formed. All these attributes then may be set aside. Each has an uncertain significance

but it cannot be employed in explanation of the *inter se* differences of this small population. Other sources of difference must be sought and of these the soil naturally comes to mind first. Now, for a pure line, equal yields from separate plots would ordinarily be accepted as proof of soil uniformity on all the plots. On this argument the seven plants whose total yields were the same, would have to be regarded as having utilised volumes of soil of identical manurial properties. But these plants display a fluctuating division of the total weights of grain and straw among their tillers (every plant had 3 ear-bearing tillers) and this fact requires further explanation. It might be argued that the volume of soil drawn on by a single plant is itself irregular. This is doubtless true but the fact is offset by what is known of the graminaceous root system. The common seminal roots, and the adventitious roots of the separate tillers, form a closely interlaced system and thus every tiller probably draws from all the parts of the total volume of soil by which the plant is nourished. It seems then, that the customary appeal to "soil differences" must here almost reach its limits of application. Present knowledge of the graminaceous plant, limited as it is in ways already described, appears to offer few other possible explanations. Light intensity has been proved in a broad way to affect tillering and it may be that shading effects play some part in determining the relative sizes of the tillers of a plant. Tillers usually arise just below the surface of the soil and if a seed be planted deep (say 3–4 inches) the tillers in the axils of the lowest foliage leaves do not develop. Thus depth of sowing—and doubtless other circumstances—influence the "order" of the tillers. There seems no reason why a second order tiller should not produce grain equally well, *ceteris paribus*, as one of the first order but there are no recorded facts by means of which to decide this issue. The functional inter-relationships of the tillers of a plant are entirely unknown and it is possibly in terms of these that an explanation should be sought.

The difficulties and irregularities encountered are, perhaps most of all, a reflection of the arbitrariness and biological indirectness of the economically important attributes, weight of grain and weight of straw. That an "attribute" is economically important and amenable to accurate measurement is no clue to its biological significance. From the degree and the varied forms of the fluctuation displayed by "weight of grain," it seems that this prime agricultural attribute is but a vague manifestation of actual physiological potentialities. The resolution of "yield" into physiological rather than algebraic components thus presents itself as of immediate practical urgency.

(To be continued.)

APPENDIX III. The Problem of "Tillering."

"Tillering" has attracted agricultural attention for centuries. Jethro Tull who explains the meaning of the word[1] is insistent upon the encouragement of tillering by "hoeing" and thereby the increasing of yield. Some of his observations suggest that he considered the practical limitations of tillering, to wit that only so many tillers are desirable as can be made to bear good ears and brought to maturity. A recapitulation of all the published results relating to tillering would be voluminous and the more helpful course seems to be a brief review of the general trend of opinion and result.

Most investigation has been directed towards one or more of the following subjects:

(i) The circumstances which influence tillering;

(ii) Inter-varietal differences in respect of tillering;

(iii) The morphology and physiology of tillering.

(iv) Various aspects of the commonly accepted "relation" between yield and tillering.

(i) The relation of tillering to circumstances of growth as governed by climate, soil, and agricultural practice has probably led to more investigation than any other aspect of the "tillering" problem. That this is so emphatically demonstrates the prevalence of the view that "good tillering means good yield." Leaving aside temporarily certain special cases, it is possible to précis most of the results into the simple statement that, *ceteris paribus*, the better the conditions of growth the greater the number of tillers per plant. In fact "tillering" is simply one of the indications of degree of success of growth. Investigations such as those of Percival[5] on wheat, of Summers[27] on rice, and of Barber[24] on the sugar-cane afford abundant detailed evidence of the influence of varying "agricultural" conditions upon tillering. Other records supplement these but it is needless to dwell on them. Percival concludes, after his elaborate investigations, that hereditary characters affect tillering but "the stem production attained by any particular kind is due in largest measure to the action of those external conditions which check or assist vegetative growth generally."

There are one or two cases worthy of special mention. Harlan[11] grew a 6-row and a 2-row barley at three different spacings, viz., $4'' \times 8''$, $4'' \times 4''$, and $4'' \times 2''$. The average numbers of tillers per plant were,

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[1] "To tiller is to branch out into many stalks, and is the Country Word that signifies the same as *fruticare*."

for the spacings in turn, 2·9 and 6·1, 2·7 and 4·5, 1·3 and 2·3. It is clear that in the case of one of the varieties (the 6-row) an increase of spacing beyond $4'' \times 4''$ led to no real increase in tillering. Percival (*loc. cit.* p. 77) relates an interesting experience with a dense-eared wheat sown in autumn upon soil in very poor heart. Areas per plant of 6, 18, and 36 square inches all gave about 1·5 to 1·8 tillers per plant whereas spacings of 72, 144, and 576 square inches gave 2·9, 4·0, and 7·0 tillers per plant. There is nothing to indicate the statistical probability of these numbers but their sequence certainly signifies. Harlan's result may perhaps be met by the hypothesis that the 6-row barley has relatively to the 2-row a very small root-range horizontally—observation of other barleys suggests that this is not improbable—but Percival's result does not appear to admit of a simple root explanation. Conjectural explanations of this kind very commonly centre upon the root system and these two sets of data suggest that much may have been lost by omitting actual observation of the root in investigations bearing upon the relation of tillering to various "conditions."

Another of the lesser-studied tillering influences is size and quality of seed sown. Information is somewhat scattered but the comprehensive literature-survey of Kidd and West (28) enables its general scope to be readily gauged. Usually the aim has been to demonstrate direct relationship between size of seed and final yield, references to tillering being more or less parenthetic. Findlay (25) worked directly upon tillering and tested the effects of large and small seeds in both oats and barley. He was satisfied that plants grown from large seeds produced a greater number of tillers than those from small. Unfortunately there is ground to suppose —as he indicates in the case of similar results with clover—that he was working with a mixed (*i.e.* non-pure-line) stock. Percival (*loc. cit.* p. 74) gives average numbers which emphatically vouch for the dependence of tillering on seed size. The relation between the N percentage of the mother seed and the number of tillers (which form ears) is clearly shown by the results which Beaven (40) gives. Within the limits which he explored, the more nitrogenous the seed the more freely did the resulting plant tiller. The extremes were, for N percentage 1·79 and 1·40 and the corresponding numbers of tillers per plant 1·67 and 1·19.

Light, perhaps, of all the suggested influences, has received the most scant attention. Percival (*loc. cit.*) for wheat and Barber for the sugar-cane (*loc. cit.* Part IV) both insist upon its importance; indeed the latter represents it as the most important of the circumstances which govern tillering (for sugar-cane in the districts in which he grew it of course).

Without special arrangements it is impossible to vary the quality or intensity of light while leaving other circumstances unaltered, and in the absence of more comprehensive and analytical investigation caution seems to be demanded as to opinions upon the exact effect of light.

(ii) Tillering as a varietal character is of great moment in plant breeding. Enough is known of the effects of soil, manuring, time of sowing, etc., upon tiller formation to permit some sort of control to be exercised in farming practice. And despite unpromising conclusions upon the relative importance of "race" and "environment" such as that quoted above from Percival, the fact that inter-varietal differences have been demonstrated ought not to be ignored. However small the influence of hereditary characters on tillering, it may yet be enough to turn the scale, to add a small percentage to average yield.

Until recently it was generally held that in crop plants outstanding desirable features could not be combined in one race. As an instance, high yield and good baking quality were supposed to be incompatible. This is now known to be fallacious. Similarly large ears and high tillering power are by many regarded as incapable of combination—in Mendelian terms, low yield and large ears show complete linkage! In all three of our common British cereals there are facts to suggest such linkages. Percival finds the rapid growing spring "vulgare" wheats to tiller better than the thicker-strawed winter ones. Among the barleys, as is commonly known, the 6-row types in general tiller less prolifically than the 2-rows. In oats, again, it is probable that Tartarian forms usually have a poorer tillering value than the "sativa" or open-panicle ones. It is gradually becoming apparent that there are partial linkages in the cereals. Their elucidation will call for much more investigation but in the meantime practical plant-breeding results are encouraging. In addition to the high-yielding strong wheats there are (on the continent) some 6-row barleys which tiller far better than the old British types. These facts suggest that preconceptions concerning incompatibility may be misconceptions and that efforts to "breed for high tillering" may yet be rewarded.

Few have made varietal studies upon tillering. Harlan (11), working with rather small numbers of plants in order to encompass many varieties, showed that in the time and manner of the formation of the first tiller, varieties did exhibit appreciable differences. But in general he found tillering not very amenable for, as he says: "the number of culms per plant seems to be a varietal character but one which is so dominated by environment as to make it impossible to determine when it is given

true expression…. In this investigation the number of tillers was recorded on over 20,000 plants without being able to discover a method of using such information for minor distinctions as was possible, for instance, with the time and method of tillering." His finding was very forcibly confirmed by growing varieties in two very different localities, a step which led him to say that "the response to geographical location is a disturbance sufficient to vitiate all close distinctions…even the groups are often reversed…but in extreme cases the effect of environment does not conceal the character." Results such as these open up possibilities of useful experiment but it will be necessary to determine not only the number of tillers but the extent of their final development. Perhaps the intangibility of so many of the investigations upon tillering has its origin in the fact that observation has been confined to number of tillers. At any rate, the work of Percival, Harlan, and Barber has shown how small is the value of this isolated observation. Summers (27) found clear evidence of varietal differences in the cultivated forms of rice with which he worked.

(iii) Admirable accounts of the morphology of tillering have been written by Barber for the sugar-cane and by Percival for the wheat plant. Morphologically, the phenomenon is not an involved one being, of course, simply the development of axillary buds accompanied by the growth of adventitious roots from the nodes. It is quite different with the physiological aspects. Knowledge of the circumstances which promote or inhibit axillary-bud development is still only general and superficial.

There is much to suggest that a cereal plant should be regarded as a colony of individuals—at any rate after the early stages—and not as a unit. Whether one tiller can profit by the activities of another has not yet been determined, for the lines and methods of transference of photo-synthetic and root-absorption products in the graminaceous plant remain unknown. Consequently it is impossible to say whether late tillers which fail to form an ear are, in relation to yield, a help or a hindrance. If their products are translocated to the ear-bearing tillers they are naturally an asset. If not, they compete for the salts and water of the soil and their contribution to the harvest is straw alone.

During the past three years experiments have been directed towards this question. They have taken the form of the removal of leaves and developing ears in various combinations. For instance, on some plants, all the ears were removed save one. This ear developed to no greater extent (as judged by average size of grain) than did corresponding ears

on comparable plants which had not been treated. In experiments of this kind the greatest caution in deduction is demanded but the general indication is that tillers are independent units. Evidence of another kind has been accumulated. The ear and straw of the successive tillers—T_0, T_1, T_2, ...—show a gradation in weight. Usually T_0 is the greatest and the others follow in sequence. In proportion of nitrogen to dry weight the order is generally the reverse of this. No special importance attaches here to the exact sequence but it is to be observed that the total nitrogen absorbed by the plant is not so distributed as to ensure a uniform concentration in all tillers at maturity. When it is recalled that the tillers are of different ages, physiologically as well as chronologically, this result is to be expected and, indeed, the individuality of the tiller seems almost self-obvious. Yield analysis can be achieved only in terms of fundamental units and should therefore, it seems, take the tiller rather than the plant, as the unit of observation.

Waldron (26) has deduced formulae for the rate of culm-formation (*i.e.* tillering) in *Bromus inermis*. His results demonstrate that tillering activity is related to the size of the plant as judged by the number of its existing tillers. It follows from this that tillering data should be derived from a population of plants selected because of the uniformity of their initial tillering. In later paragraphs it appears that such a selection seems to offer the only chance of reasonably restricting the fluctuation of other variables and a method of procedure is suggested in § VIII of the text.

(iv) The relation between yield and tillering as commonly conceived, is a statistical problem. For its solution data of two distinct categories are requisite. It is necessary first to know the form of the relationship for different populations of one pure line grown on a variety of soils and in successive years. Then again, comparisons must be drawn among suitably grown populations of different pure lines. From the considerable body of more or less relevant published results very little can be extracted which is of critical value in either category. The most direct evidence appears to be that from various chess-board trials of yield which have from time to time been made. Their results have not been published in detail. Certain facts stand out from the 1911–14 Cambridge Barley Trials. Correlations were evaluated among the following variables:

(α) Yield per plot.

(β) Number of surviving plants per plot.

(γ) Average number of ears per plant for the plot.

(δ) Average weight per ear for the plot.

(ϵ) Number of ears per plot = (β) × (γ).

For all the varieties in turn, (α) was more highly correlated with (ϵ) than with any other variable. Next in order was the correlation between (α) and (γ). This, however, was not high, the reason being readily apparent from an inspection of the data. On some of the plots for which (γ) was high, (α) was low and for these it was found that (β) also was low. Many plants had died and, although the survivors had tillered very freely—thus making (γ) high—this had failed to compensate for the loss in number of plants, a loss which had induced the low value of (α).

Inter-varietal comparisons revealed complexities. In one year variety A might have a greater yield than B and statistically the difference was attributable to the superiority of A in respect of (γ). Next year A might again out-yield B but statistically for a different reason viz., superiority in respect of (δ). Complexities of this kind are to be expected since variables (β), (γ), and (δ) are known to be interdependent. Although it is extremely difficult to assess the relationship of these separate variables to yield per unit area, it seems justifiable to assume, from the general trend of the chess-board results, that as among populations of one pure line or as between different pure lines, tillering is fundamentally related to yield. At present neither the results here described nor any others appear to permit the extension of this simple generalisation.

The "tillering problem" remains obscure and for its further elucidation investigation upon the following matters seems to be essential:

(α) Methods of measuring tillering at various stages with an accuracy greater than that customarily achieved.

(β) The relation between early tillering and final ear formation.

(γ) Inter-tiller relationship for the single plant in regard to time of formation and final development [since yield per acre depends upon the sizes of the tillers as well as their number.]

(δ) The practicability of restricting experimental populations to plants grown at uniform spacing [the chess-board correlations clearly show the obscuring effects of spatial fluctuation.]

Essays on these lines have taken the form of investigations upon early tiller formation [§ VII of the text], inter-tiller relationship [§ IX] and the relation of tillering to yield per plant [§ XI].

APPENDIX V. Time of Flowering and Time of Ripening; Definitions, Genetic and Other Evidence of the Reliability of these Two Characters; their Possible Relation to Yield.

In general practice it is safe and simple to classify cereal varieties as early or late in regard to flowering and ripening. For experimental purposes the extent of earliness or lateness must be precisely measured and flowering and ripening have to be defined. That, as between varieties, lateness implies heavy yield—or, better, that earliness prohibits it—is very generally held. To examine various definitions, to seek for evidence of a relationship to yielding power, and to assess the reliability or constancy of these two time characters, a brief survey of experimental results is desirable. Genetic results are the most valuable for they have involved the most critical tests.

Biffen[31] in a wheat cross (Polish × Rivet) found very clear evidence of simple 3 : 1 segregation in regard to ripening of the grain. He found, too, that the ripening character was quite independent of the general "habit" of the plant. For example, among the F_2 plants of obvious Polish habit, there was a regular (1 : 2 : 1) distribution of the three degrees of ripening which he distinguished.

Caporn[33] in an oat cross, defined ripeness as the disappearance of the last traces of green from the tips of all the paleae. He does not appear to have dealt with separate tillers—some of which may lag far behind in the oat—but to have waited until all were ripe before accepting a plant as "ripe." His observations were confined to F_3 families and the parents. "Period of ripening" was the form of measurement and it was defined, for any population (*e.g.* an F_3 family or a population of a parental strain) as the period between the dates of ripening of the first and last plants to ripen. Of the 106 F_3 families raised, all had earlier periods than the late parent while all but two had later periods than the early parent. The results were obscure but it was concluded that time of ripening was "Mendelian" and that possibly three factors governed it. The following passage is of interest in connection with yield: "the tillering of late forms is always good; that of early, very poor. Owing to the concentration of growth among early plants into one or two panicles only, these are generally bigger and bear better grain than those of late plants; but this advantage does not compensate for the diminished yield due to the small number of heads. There is thus an inevitable sacrifice of crop when it is attempted to render a late kind early." This view, unsupported by data, demands cautious acceptance.

Freeman [34] in a wheat cross, investigated "date of first head," *i.e.* of the emergence of the first ear of the plant from the top sheathing leaf. His ultimate conclusion was that "all the phenomena can be explained by assuming that heading-date is governed by three or more Mendelizing unit factors. No attempt was made to determine the number of factors but there were reasons for supposing it to be rather large." From this conclusion it might be inferred that ' date of first head" was a character exhibiting an exceedingly complicated mode of inheritance. But certain facts must first be weighed, viz. (i) only 230 of the 2546 F_2 plants were grown on to F_3 and these were definitely "selected" ones, (ii) the parental populations were not pure lines but "mass selections," (iii) deductions were based upon the principle that if segregation is taking place, the mean value of the coefficient of variation (σ/M) for F_3 families will be less than that for F_2 families since, in a self-pollinating plant the "percentage of heterozygosity" will decrease from F_2 to F_3 to F_4 and so on. An observed decrease in σ/M will thus imply the operation of segregation. Not much was to be expected, perhaps, from such a non-analytical method and the more so that σ for some of the F_3 families was based upon 12–15 plants only.

Thompson [37] investigated for ten wheat crosses the inheritance of:

"ripening period" = number of days from sowing to ripening,

"heading period" = number of days from sowing to heading.

It is not clear whether the first or all of the heads of a plant were used to decide its ripeness. ["A plant was called ripe as soon as the central kernels reached the dough stage."] For every cross, the frequency distribution of "ripening period" was plotted for the complete F_2 and proved to be uni-modal. With "time of heading" the same procedure led to the same result. No precise conclusion was reached concerning inheritance. Some of the F_2's were small (the ten crosses contained from 102 to 541 plants per F_2) and no F_3's appear to have been grown. Here again, therefore, the data cannot be accepted as evidence of complexity of inheritance of the two time characters.

Hoshino [35] made studies of peas and, less completely, of rice; and since these rank as the classical genetic investigations on time characters. they must be briefly considered. His definition of time of flowering was:

Let p = number of days from sowing to flowering,

q = number of days from "sprouting" to flowering.

"Flowering" implied the opening of the vexillum of the first flower. Then time of flowering = $(p + q)/2$.

In English-grown cereals the interval between sowing and "sprouting" (emergence of the plumule from the soil) is very constant under the conditions of careful experiment so that, for them, this form of definition would have no advantage over p or q alone. His results with peas suggested that two factors controlled flowering period and, further, that hybridization resulted in "gametic contamination." Further, he found a coupling (linkage) between one of the flowering factors and a factor for red colour [*cf.* Lock and Tschermak]. For rice a three-factor hypothesis was suggested. Clearly, the case was not a simple one, and Castle (38) has suggested an alternative explanation based upon a single factor and the operation of extensive "gametic contamination." These facts notwithstanding, Hoshino's work puts flowering period on the same plane of experimental practicability in genetic work—and therefore of general biological reliability—as a great number of the accepted plant characters.

Keeble and Miss Pellew (32) for sweet peas found evidence of "coupling" between lateness and thickness of stem and Cowens (36), in *Hyoscyamus niger*, found the biennial habit to be simply dominant to the annual.

These genetic studies strongly suggest that ripening and flowering time factors are, within the limits of present knowledge of "plant characters," constant and reliable for any given variety. In furtherance, the evidence obtained from systematic studies, may be considered.

Percival (5) found that the period from sowing to "earing" in wheat was affected by the actual date of sowing especially for sowings made in July and other unusual sowing months. But the actual date of earing showed a much closer approach to constancy for a great range of sowing times. Indeed, for every variety sown year after year on a particular date, the date of earing over a period of years was so constant as to be a diagnostic character. The numerical data adduced lacks the finality which evidence of probability (probable errors, etc.) would have afforded, but its general trend is unmistakeable.

Harlan (39), for barley, found the emergence of the awn from the top leaf sheath to be "a very accurate index of the stage of development of the spike." To mark equally developed spikes he selected the criterion of an emergence of $\frac{1}{2}-\frac{1}{4}$ inch. In another investigation [*ibid.* (11)] he emphasised the superiority of awn emergence as a "character" to either "heading" or "ripening."

The evidence which has been examined appears to make clear that the two time attributes in cereals are sufficiently constant in varieties to warrant their employment as characters in investigation. Ear emergence—suitably measured—appears to be more constant than

ripening but the two are closely related. When experimental require-
ments permit, it is better to base deductions upon actual dates than
upon periods between two dates (*e.g.* sowing and ripening).

In the literature of this subject it is difficult to find evidence as to
the relation of the times and periods of flowering and ripening to yield
(as between varieties). And similarly, the inter-relations of the tillers
of a single plant in respect of these two processes, are not usually
mentioned. To a great extent, these two subjects are really, it seems, one.
For, particularly in oats, there are commercial varieties which seem,
quite characteristically, to flower and to ripen their main-axis panicle
considerably before their side (tiller) ones. In consequence, since oats
must be cut before dead ripeness, the tiller panicles often contribute very
poor and very unripe grain. At present it is not possible to demonstrate
that the later of such tillers are positively harmful but it is not un-
reasonable to suppose that the yield might have been higher if they had
not been formed and thus had not competed with the bigger tillers. Not
only the flowering times of the tillers but their grain and straw production
as well, need to be studied in different varieties. But the flowering of
the individual plant is very much more constant than its grain production
and for that reason its investigation—for separate tillers—must be
regarded as an important part of yield-power investigation on the
analytical basis.

A belief in the inevitable association of earliness and low yield
[*cf.* Caporn (above)] cannot be upheld by precise observations and until
these have been made, judgment ought to be reserved.

Key to Tables I–XI for P and A showing the Bed, Spacing and Date
to which every table applies.

Tables having an "A" in their serial numbers give both numbers of
leaves (x) and of tillers (y) in the form ($x.y$).

Table I is derived from 1 (A) and so on for II, III and IV.

	Bed number and sowing interval			
Date	Bed 5 = 4″	Bed 6 = 4″	Bed 7 = 2″	Bed 8 = 2″
21. iv. 21	I and I A	II and II A	III and III ·	IV and IV A
28. iv. 21	· — ·	... ·	... ·	V
7. v. 21	VI	—	—	VII
25. v. 21	VIII	IX	X	XI

Tables I–IV. For P and A. The Frequency Distribution of Number of
   Tillers for Plant on 21. iv. 21. Frequencies are given as percentages.
   At the head of every table is shown the number of plants observed,
   at the foot the mean (with standard error) number of tillers per plant.

Table no. ...	I		II		III		IV	
Variety ...	P	A	P	A	P	A	P	A
No. of plants	139	167	161	171	389	443	382	355
No. of tillers per plant								
0	25·90	28·75	15·53	21·05	49·88	32·72	35·32	32·38
1	31·75	36·53	35·40	41·81	37·03	44·01	44·75	45·06
2	40·29	34·13	48·45	36·83	12·86	23·03	19·37	22·24
3	2·16	0·59	0·62	0·58	0·26	0·23	0·52	0·28
Mean no. of tillers per plant	1·18	1·06	1·34	1·17	0·63	0·90	0·85	0·90
Standard error of	±0·071	±0·062	±0·058	±0·058	±0·036	±0·036	±0·038	±0·039

Table I A–IV A for P and A. The Frequency Distribution of Numbers
   of Leaves (x) and of Tillers (y), (expressed in the form $x.y$) on
   21. iv. 21. Frequencies are given as percentages and the number of
   plants involved is shown at the head of every table.

Table no. ...	I A		II A		III A		IV A	
Variety ...	P	A	P	A	P	A	P	A
No. of plants	139	167	161	171	389	443	382	355
$(x.y)$								
2·0	0·72	—	—	0·58	0·51	0·45	0·26	—
3·0	16·55	22·16	11·18	12·28	38·31	28·21	29·83	28·16
3·1	1·54	1·20	—	2·34	5·66	6·09	1·57	4·79
3·2	—	—	—	0·58	—	0·23	—	0·28
4·0	8·63	6·59	4·35	8·19	11·06	4·06	5·23	4·22
4·1	30·21	35·33	35·40	39·17	31·37	37·92	43·18	40·27
4·2	40·29	34·13	48·45	36·25	12·86	22·80	19·37	21·96
4·3	2·16	0·59	0·62	0·58	0·26	0·23	0·52	0·28

No. of tillers and no. of leaves per plant

Table V for 28. iv. 21 and Tables VI and VII for 7. v. 21 for P and A (see note at head of Tables I–IV above).

Table no. ...	VI		VII		V	
Variety ...	P	A	P	A	P	A
No. of plants	84	96	238	186	249	182
No. of tillers per plant 0	—	—	—	0·54	1·60	1·10
1	3·57	1·04	1·26	2·69	18·02	16·48
2	10·71	9·36	27·73	17·75	40·04	32·96
3	16·66	7·28	19·32	33·33	29·62	35·71
4	16·66	26·00	30·67	22·59	10·00	12·64
5	27·37	23·92	13·45	15·06	—	1·10
6	15·47	13·52	5·90	6·99	0·40	—
7	9·52	15·60	1·26	1·07	—	—
8	—	2·08	0·42	—	—	—
9	—	1·04	—	—	—	—
Mean no. of tillers per plant	4·38	4·81	3·52	3·53	2·30	2·46
Standard error of mean	±0·173	±0·163	±0·085	±0·095	±0·061	±0·073

Tables VIII–XI for 25. v. 21 for P and A (see note at head of Tables I–IV above).

Table no. ...	VIII		IX		X		XI	
Variety ...	P	A	P	A	P	A	P	A
No. of plants	82	93	93	118	275	247	200	156
No. of tillers per plant 0	—	—	—	—	1·09	2·03	0·50	0·64
1	1·22	2·16	3·23	2·54	5·82	3·65	1·50	3·21
2	2·44	3·24	2·15	—	25·84	23·49	23·50	17·31
3	3·66	8·64	4·30	7·62	28·39	26·33	16·00	30·77
4	15·86	16·20	4·30	10·16	24·39	25·92	26·50	21·15
5	21·96	23·76	21·50	20·33	10·92	13·77	19·50	16·67
6	17·08	19·44	17·20	24·56	3·64	3·24	6·00	9·62
7	8·54	15·12	19·35	13·55	—	1·62	5·50	0·64
8	12·20	8·64	16·13	10·16	—	—	1·00	—
9	10·98	—	7·53	5·93	—	—	—	—
10	2·44	2·16	2·15	2·54	—	—	—	—
11	2·44	1·08	1·08	2·54	—	—	—	—
12	1·22	—	1·08	—	—	—	—	—
Mean no. of tillers per plant	6·15	5·41	6·27	5·98	3·16	3·33	3·83	3·60
Standard error of mean	±0·243	±0·193	±0·216	±0·185	±0·069	±0·087	±0·109	±0·108

Table XII (which also serves as a ground-plan of the beds). The means (nos. of tillers per plant) of Tables I–XI for P and A showing the Bed number, sowing interval, and date.

Date	Bed 5 = 4″		Bed 6 = 4″		Bed 7 = 2″		Bed 8 = 2″	
	P	A	P	A	P	A	P	A
21. iv. 21	1·18 ±0·071	1·06 ±0·062	1·34 ±0·058	1·17 ±0·058	0·63 ±0·036	0·90 ±0·036	0·85 ±0·038	0·90 ±0·039
28. iv. 21	— —	— —	—	—	—	—	2·30 ±0·061	2·46 ±0·073
7. v. 21	4·38 ±0·173	4·81 ±0·163	—	—	—	—	3·52 ±0·085	3·53 ±0·095
25. v. 21	6·15 ±0·243	5·41 ±0·193	6·27 ±0·216	5·98 ±0·185	3·16 ±0·069	3·33 ±0·087	3·83 ±0·109	3·60 ±0·108

Table XIII for P and A, Bed 7. Frequency Distribution of numbers of ears ripened by plants which, throughout, were grown under "uniform conditions." Frequencies are given as percentages.

	No. of ears ripened per plant								No. of plants
	<3	3	4	5	6	7	8	9	
P	41·75	36·59	14·95	5·15	1·03	—	0·52	—	194
A	46·06	32·12	15·15	5·45	0·61	—	—	0·61	165

Table XXIII. P and A. Beds 6 and 7 (at 4-inch and 2-inch spacing). Frequency distribution of date of emergence of main axis (T_0) ear for all plants (excluding, as usual, outside and other exceptionable plants). Frequencies are given as percentages.

Bed no. and spacing	Variety	No. of plants	Dates					
			11. vi.	13. vi.	15. vi.	17. vi.	19. vi.	Later
Bed 6 4-inch	P	71	4·22	38·03	22·53	23·94	9·86	1·41
	A	110	32·73	29·01	19·10	11·82	3·64	3·64
Bed 7 2-inch	P	267	2·25	45·69	27·71	18·73	4·49	1·12
	A	230	4·34	57·39	19·57	13·04	1·74	3·91

Table XXIV. P and A. Bed 7 (2-inch spacing). Frequency distribution of dates of emergence of the ears of T_1 and T_2 for plants whose T_0 ear emerged on 13. vi. Frequencies are given as percentages.

Variety	No. of plants	Ear	Dates					
			11. vi.	13. vi.	15. vi.	17. vi.	19. vi.	Later
P	122	T_1	—	32·79	40·16	16·39	4·10	6·56
	122	T_2	—	1·64	21·31	39·34	6·56	31·15
A	132	T_1	—	31·06	31·81	21·97	6·06	9·09
	132	T_2	—	5·30	19·70	37·12	5·30	32·58

Table XXV. P and A. Bed 7 (2-inch spacing). Frequency distribution of date of emergence of T_0 for plants which were 3·0 (*i.e.* had 3 leaves and no tillers) on 21. iv. and for those which were 4·1 (*i.e.* had 4 leaves and 1 tiller) on 21. iv. Frequencies are given as percentages.

Condition on 21. iv.	Variety	No. of plants	Date of emergence of T_0					
			11. vi.	13. vi.	15. vi.	17. vi.	19. vi.	Later
3·0	P	103	0·97	33·98	26·21	26·21	7·77	4·85
	A	81	1·23	34·57	23·46	27·16	6·17	7·41
4·1	P	85	3·53	61·17	23·53	9·41	1·18	1·18
	A	81	2·47	76·54	17·28	2·47	—	1·23

Table XXVI. P and A. Bed 7 (2-inch spacing). Frequency distribution of number of ears per plant which emerged between 11. vi. and 19. vi. for plants which were (3·0) or (4·1) on 21. iv. Frequencies are given as percentages.

Condition on 21. iv.	Variety	No. of Plants	No. of ears per plant				
			0	1	2	3	4
3·0	P	103	4·85	34·95	34·95	25·24	—
	A	81	16·05	32·10	33·33	17·28	1·23
4·1	P	85	1·18	3·53	25·88	51·76	17·65
	A	81	1·23	2·47	29·63	49·38	17·28

Table XXVII. P and A. Bed 7 (2-inch spacing). Frequency distribution of date of emergence of main axis (T_0) ear for plants which ripened 3 or more ears at harvest.

Variety	No. of plants	Date of emergence of T_0					
		11. vi.	13. vi.	15. vi.	17. vi.	19. vi.	Later
P	113	1·77	53·09	30·09	9·73	5·31	—
A	89	2·25	66·31	19·10	12·36	—	—

Table XXVIII. P and A. Bed 7 (2-inch spacing). Frequency distribution of number of leaves and number of tillers (expressed in the Table as $x.y$) on 21. iv. for plants whose main axis (T_0) ear emerged on 13. vi. Frequencies are given as percentages.

Variety	No. of plants	Number of leaves (x) and tillers $y = (x.y)$ on 21. iv.							
		2·0	3·0	3·1	3·2	4·0	4·1	4·2	4·3
P	122	—	28·69	3·28	—	10·66	44·26	13·11	—
A	132	0·75	18·94	6·82	—	4·54	45·45	22·73	0·7?

Table XXIX. P and A. Bed 7 (2-inch spacing). Frequency distribution of number of tillers per plant on 25. v. for plants whose main axis (T_0) ear emerged on 13. vi. Frequencies are given as percentages.

Variety	No. of plants	No. of tillers on 25. v.							
		0	1	2	3	4	5	6	7
P	122	—	2·46	22·95	33·61	24·59	11·48	4·92	—
A	132	--	2·27	20·45	27·27	28·77	17·42	3·03	0·75

Table XXX. Frequency Distribution of Dates of Emergence of T_1 and T_2 for plants used in tiller-by-tiller weighings.

Variety	Tiller no.	Date of emergence				No. of plants
		13. vi.	15. vi.	17. vi.	19. vi.	
P	T_1	9	13	2	—	24
	T_2	1	2	17	4	24
A	T_1	13	11	7	—	31
	T_2	1	6	21	3	31

Table XXXI. The Weights of Grain (Yields) of the P and A Plants which are described in § IX of the text. Weights are in centigrammes.

Plumage

210	237	280	327	373	414	443	491
216	242	288	329	375	425	445	492
222	255	300	—	386	—	452	511
—	—	—	—	398	—	461	—

Archer

267	282	317	351	409	436	461	487
—	285	318	358	411	437	467	497
—	298	320	361	413	438	—	499
—	306	322	371	—	441	—	—
—	—	330	371	—	441	—	·—
—	—	343	—	—	442	—	—
—	—	—	—	—	442	—	—

Table XXXII. The Full Data for One Group of A Plants.

G = weight of grain ⎫
S =   ,,   straw ⎬ per plant
R =   ,,   rachis ⎭
n = number of grains
B = weight of odd pieces (detached) and infertile tillers.

T_0 = main axis ⎫
T_1 = first side tiller ⎬ of the plant
T_2 = second ,, ,, ⎭

Weights are in centigrammes unless otherwise stated

G plant total		436	437	438	441	441	442	442
G as per cent. of total	T_0	36·7	39·8	37·2	34·7	33·6	38·5	43·4
	T_1	31·9	37·3	34·9	35·6	32·9	35·5	31·7
	T_2	31·4	22·9	27·9	29·7	33·5	26·0	24·8
$S + R$ plant total		393	356	369	387	367	439	406
S as per cent. of total	T_0	40·8	46·0	38·9	39·11	42·7	42·1	38·0
	T_1	29·6	36·5	34·8	34·63	28·6	27·8	28·6
	T_2	29·6	17·5	26·3	26·25	28·6	30·0	33·4
$G/(S + R + B + G)$ plant...		0·521	0·515	0·519	0·490	0·526	0·497	0·434
$G/(S + R)$	T_0	0·987	1·061	1·139	1·013	0·948	0·918	1·222
	T_1	1·208	1·263	1·180	1·171	1·367	1·276	1·196
	T_2	1·181	1·587	1·257	1·284	1·409	0·877	0·833
n plant total		91	86	85	83	85	90	90
n tillers..............	T_0	31	32	30	28	28	31	33
	T_1	30	31	29	29	28	31	29
	T_2	30	23	26	26	29	28	28
G/n plant		4·79	5·08	5·15	5·31	5·19	4·91	4·91
G/n tillers............	T_0	5·16	5·43	5·43	5·46	5·28	5·48	5·81
	T_1	4·63	5·25	5·27	5·41	5·17	5·06	4·03
	T_2	4·56	4·34	4·69	5·04	5·10	4·11	3·92
Date of emergence of ear	T_0	13. vi.	13. vi.	13. vi.	13. vi.	13. vi.	13. vi.	13. vi.
	T_1	13. vi.	13. vi.	13. vi.	13. vi.	13. vi.	13. vi.	15. vi.
	T_2	17. vi.	19. vi.	15. vi.	15. vi.	13. vi.	17. vi.	17. vi.

Table XXXIII for the 24 selected P plants showing weight of grain (G) borne by tillers and total weights borne by whole plants. The plants are arranged in order of magnitude of T_0. Unit of weight = 1 centigramme.

T_0	T_1	T_2	Plant	T_0	T_1	T_2	Plant
93	83	46	222	154	167	140	461
93	89	38	220	158	141	126	425
95	62	59	216	166	109	98	373
101	96	83	280	171	136	145	452
104	91	60	255	172	129	85	386
110	77	50	237	177	118	103	398
110	67	65	242	178	172	141	491
114	121	92	327	187	156	102	445
120	110	70	300	187	168	156	511
121	106	61	288	190	131	122	443
132	105	92	329	198	150	66	414
149	136	90	375	207	167	118	492

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FURTHER INVESTIGATIONS INTO THE CHANGES WHICH OCCUR DURING THE ENSILAGE OF A GREEN CROP.

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INTRODUCTION.

IN recent communications accounts have been given of investigations which were designed to throw light on the nature and extent of the changes which occur when a green crop is converted into silage and to furnish information regarding the factors which influence the course of the fermentation and determine in a large measure the quality of the silage.

Amos and Williams[1] discussed these factors at some length, directing attention especially to the temperature of fermentation associated with the production of the different types of silage.

In further work Amos and Woodman[2] demonstrated that the moisture-content of the ensiled fodder exerted an important influence not only on the production of different types of silage, but also on the extent of the changes affecting the individual constituents of the crop.

It has long been recognised, from general experience in the practice of ensilage, that the type of silage produced is to a marked extent determined by the stage of maturity of the ensiled crop. In the case of green "fruity" oat and tare silage, for instance, there can be little doubt that its production depends upon the ensiling without wilting of an oat and tare crop in the early stages of maturity, when the oats are in milk and the tares are between the stages of full flower and half-formed seeds. This result has been confirmed in scientific tests by the writers and also by the collected experience of makers of silage.

Experience further indicates that if the ensiled crop is too immature and succulent, the result may be the production of the so-called "sour" silage. Instances of this are cited by Amos and Williams[1]. In one case, over a period of several years, maize was grown and ensiled in a tower

silo. The variety grown, however (American Horse Tooth), failed to ripen sufficiently for ideal silage purposes, the crop rarely getting beyond the flowering stage. Under these conditions the silage was invariably "sour" with a pungent clinging smell.

On the other hand, if the crop is more mature than is required for green "fruity" silage (*i.e.* if the oats are past the milk stage and the tares are well seeded) an acid brown or yellowish-brown silage appears frequently to be produced. This possesses an acid yet pleasant odour, due probably to acetic and propionic acids, and certainly not possessing the unpleasant smell of butyric acid associated with "sour" silage.

Amos and Williams further quote an isolated case where over-ripe and wilted oats and tares containing much charlock gave rise to a musty smelling silage.

It would thus appear that the combination of experience and experiment points to the following generalisations in regard to the influence of the maturity of crop on the quality of silage:

1. "Sour" silage results from ensiling very immature and succulent crops.

2. Green "fruity" silage from crops in early to medium maturity.

3. "Acid brown" silage from fairly mature crops.

The experiments about to be described were designed partly with the object of testing these conclusions and partly with a view to ascertaining the extent of the changes and consequent losses sustained by the same crop when ensiled at different stages of maturity.

For the purpose of the tests, a crop of oats, tares and beans was selected as being one which was likely to stand well. The crop was cut at three different stages of maturity and ensiled as quickly as possible in three of the small experimental wooden silos described in a previous communication(2). So far as possible, no other factor save that of maturity was permitted to vary on the three occasions; thus the crop was reasonably free from superficial moisture in every case; in all three cases it was cut in the early morning and chaffed and ensiled immediately; furthermore, in no case was the crop damaged by decomposition of the basal foliage, a condition which is known to prejudice the quality of the silage.

DESCRIPTION OF THE CROP.

The seeding of the crop consisted of 2 parts oats, 1 part tares and 1 part beans, sown on October 6, 1921, at the rate of $3\frac{1}{4}$ bushels of the mixture an acre by means of an ordinary corn drill. All the seeds

germinated and grew well and on June 21, 1922, when several plots were measured, gave a yield of 11·75 tons of green crop per acre (3·4 tons dry matter per acre).

The three dates of cutting the crop were June 14, June 23 and July 12. June 14, when the first silo was filled, was preceded by three weeks of drought, though in the previous afternoon and evening there had been a drizzle of rain, which, however, ceased before morning. The crop was very slightly wet with this rain when cut. After chaffing, mainly owing to sap, it was very wet to handle. At this stage the largest pods on the beans were 1½ inches long and the stems were green and succulent; the tares were in full flower, the largest pods being only ½ inch long; the oats had just flowered. There was a certain amount of weed in the crop, especially Venus Comb (*Scandix pecten*), Corn Buttercup (*Ranunculus arvensis*) and Black Bent Grass (*Alopecurus agrestis*) from which the seeds, though full-grown, had not begun to drop. The crop at this stage would generally be considered immature for silage.

The second filling on June 23 was preceded by fine, dry and cool weather for the most part, but showers fell during the previous night. There was not much rain upon the crop, however, when carted, though when chaffed it felt rather wet. The crop, in consequence of the cool weather, had not progressed very rapidly in maturity. The largest bean-pods were now well grown and contained half-grown seed; the largest tares-pods were 2″ in length, but were very scanty owing to damage by midge bites; the oats were just arrived at the milk stage. The weeds mentioned previously were again prominent, but as yet had shed little or no seed. The crop at this stage might be regarded as ideal for the purposes of silage.

The weather previous to the cutting of the crop for filling the third silo had been cool and showery, with high winds prevailing. Consequently the crop was somewhat badly laid when cut, but fortunately had not begun to decompose at the base. At this stage, the glumes covering the oats were beginning to change colour from green to yellow, and the grain was passing out of the milk stage. The beans had lost much of their foliage, the pods being full grown and the seeds beginning to show blackening on the hilum. The tares-pods were full grown and contained seeds from half to three parts full grown. Many of the weeds previously mentioned had ripened seed before this time, which had either been shed before cutting or was scattered by the act of cutting. The crop at this stage would usually be considered past its prime for silage.

In addition to the experiments which were made in the small experimental silos, a sample bag containing a portion of the crop cut on June 30 was placed in the upper half of the commercial silo and surrounded by material cut at the same time. At this stage, the oats were all in milk, the tares were showing useful pods with seeds a quarter grown and the beans were proportionately advanced.

Taking the stage of development of the oats as the index, it will be seen that the crop was cut and ensiled at the following stages of maturity:

June 14. Oats had just flowered.

June 23. Oats arrived at milk stage.

June 30. Oats all in milk.

July 12. Glumes changing from green to yellow, and grain passing out of milk stage.

FILLING OF SILOS AND SAMPLE BAGS.

In every case, the crop was carted immediately after cutting and chaffed by an ensilage cutter. Samples of the chaff were well mixed in a heap on a concrete floor and the heap divided into three parts. From one part the analytical sample was taken and from each of the other parts sample bags were filled. The latter were made of jute and measured 3 feet in length and 1 foot 9 inches in width. When filled, they contained from 35 to 50 lb. of green fodder, depending on the moistness of the crop. They were weighed empty and again when filled.

In filling the small wooden stave silos, which were 6 feet high and 4 feet in diameter, the chaffed crop was put in and trodden down till a height of 2 feet was reached, and the first sample bag was then carefully placed in position with a maximum thermometer enclosed in a short length of gas-pipe immediately below. The filling and treading again proceeded till a height of 4 feet was reached, when the second sample bag with maximum thermometer was placed in position as before.

The filling was again continued till the top was reached, when an extension of iron sheeting was fixed to the silo, so that the latter could be filled 9 to 12 inches above the top. After this, a layer of 6 to 8 inches of earth was thrown on the top, and within a few days the material had settled down into the silo and the extension was removed.

OPENING OF SILOS.

The small silos were opened successively on November 7, 14 and 21 in the same order as that in which they were filled.

Silo 1. Contained only 2 inches of spoilt material on top. The silage

from the two sample bags was still very wet and possessed a greenish-brown colour. That in the top bag had a fresh smell when first exposed with a slight suggestion of "fruitiness," but this soon gave place to a more pungent and less pleasant odour. That in the lower bag possessed a fresh odour which was in no sense fruity. The maximum thermometers did not rise beyond 20° C.

Silo 2. Contained 9 inches of waste on top. The material in both bags was dark olive green in colour and possessed a very pleasant "fruity" odour. The maximum thermometers registered 20° C. in the upper sample and 21·5° C. in the lower sample.

Commercial silo. Contained two sample bags. The upper bag containing material cut on June 30 was removed from the silo on December 8 and was found to contain green "fruity" silage of good quality. The maximum thermometer registered 28·25° C. The sample bag in the lower half of the silo contained a sample of the same material as was filled into Silo 2. It was taken from the silo on April 16, 1923, and its contents proved to be excellent silage of the green "fruity" type. The maximum thermometer registered 25° C. As a matter of fact, the bulk of the silage from the commercial silo was a good quality green "fruity" silage, the silo having been filled with green crop cut between early and medium maturity.

Silo 3. Contained a 6-inch layer of completely spoilt material on top. Below this there was a further 3 inches of slightly mouldy material, and occasional spots of mould persisted to the depth of the upper sample bag. The contents of the latter were brown in colour, displaying numerous inconspicuous spots of white mould. The smell was musty and the material was sticky to the touch. The maximum thermometer registered 35° C. The lower bag, more efficiently protected from air by reason of its greater depth, contained good silage, which was fairly dry to handle. The colour was brown and the smell slightly acid, but quite pleasant. The maximum temperature recorded was 24·5° C.

Table I records the dry weights of green crop placed in the different sample bags and also the dry weights of the resulting silage. The moisture contents of green crop and silage are also given together with the percentage losses of dry matter resulting from conversion of the samples of green crop into silage.

From the foregoing description of the contents of the sample bags, it is at once evident that the types of silage produced from the differently matured samples of green crop were markedly distinctive.

The quality of the silage obtained from the very immature and

succulent crop was rather better than had been anticipated. The unpleasant characteristics usually associated with really "sour" silage were not noticeable to any marked extent, and this may have been due to the fact that the crop was ensiled without wilting.

Table I.

No. of silo			Silo 1		Silo 2		Silo 3		Commercial silo	
No. of bag			1	2	3	4	7	8	5	6
Position in silo			Lower	Upper	Lower	Upper	Upper	Lower	Lower	Upper
% moisture as ensiled			77·95	77·95	73·56	73·56	69·25	69·25	73·56	76·75
% moisture after ensiling			77·96	78·14	74·16	74·39	71·90	70·58	71·49	74·44
Dry matter as ensiled (oz.)			187·0	190·3	187·7	168·4	174·0	186·7	177·4	181·8
Dry matter after ensiling (oz.)			168·8	167·9	170·8	153·9	154·3	175·9	143·4	168·4
% loss of dry matter			9·7	11·8	9·0	8·6	11·3	5·8	19·2	7·4

The character of the silage obtained from bags 3, 4, 5 and 6 was in accordance with expectation. The ensiling without wilting of a crop cut between early and medium maturity generally gives rise to the best type of silage, namely, green "fruity" silage.

The material obtained from the upper bag of Silo 3 could not properly be described as silage, but it is a condition which may be expected when over-mature crops are ensiled in a dry condition and air imperfectly excluded. In practice under such circumstances, water may be added with advantage at the time of ensiling.

The silage produced in the lower bag of Silo 3 demonstrated the truth of the statement made in the introduction, that fairly mature crops ensiled in a dry condition generally give rise to the "acid brown" type of silage.

It is interesting at this stage to compare the previous year's results [2] in connection with the effect of varying green crop moisture-content with the results of the present investigation into the effect of stage of maturity.

1. Crop cut in early maturity and ensiled without wilting gave rise to green "fruity" silage (both investigations).

2. Crop cut in early maturity and allowed to dry by wilting for 24 hours before ensiling gave rise to "acid brown" type of silage (previous investigation).

3. Crop allowed to become fairly mature and ensiled without wilting also gave rise to "acid brown" silage (present investigation).

The combined results suggest that the effect of allowing the crop to become fairly mature is connected in some measure with the drying out of the crop which goes on during the process.

METHODS OF ANALYSIS.

The methods of analysis were in the main identical with those already described in an earlier communication (2). It was, however, found desirable to modify in one or two particulars the method of dealing with the silage extracts. In previous work it was noticed that the percentage of non-volatile organic acids in the silage calculated in terms of lactic acid from the titration results of the alcoholic liquors was frequently appreciably greater than would be anticipated from the amount of ether extract contained in the silage after drying at $100°$ C. An examination of the method as then used revealed an explanation for this discrepancy. The non-volatile organic acids were not estimated by direct experiment but were calculated by difference in the following manner:

Non-volatile organic acids = Total titration value − (amino acids
+ volatile organic acids).

As, however, the total titration value includes the titration value of carbon dioxide, whereas this latter figure escapes inclusion in the value for the volatile organic acids, it follows that the figure for non-volatile organic acids must be on the high side owing to its representing in actuality lactic acid + carbon dioxide. The figure also includes combined lactic acid, whereas treatment with ether only extracts free lactic acid.

It was decided, therefore, to estimate the non-volatile acids directly by titrating the residue after distilling off the volatile acids in steam. By this means, results in good harmony with those recorded by Dox and Neidig (3) for the relation between volatile and non-volatile organic acids in silage were obtained.

The method for determining the amino acids in the alcoholic liquors was also slightly modified. Instead of using the indirect method involved in Foreman's (4) method of determining volatile bases, the direct method devised by this investigator was employed by removing the alcohol from 100 c.c. alcoholic liquor in a current of steam blown through for 10–15 minutes. The residue was made up to 200 c.c. with water and aliquot portions were titrated with and without the presence of alcohol under the conditions laid down by Foreman. The difference between the two titrations when diminished by the amount of volatile bases gave the figure for the amino acids. Slightly higher values were obtained by this method than by the indirect method. Preliminary tests proved that the volatile bases and amino acids are not affected by the short steam distillation.

In making up the alcoholic silage liquors, 100 c.c., and not 150 c.c. as formerly, of the aqueous extract were diluted to 500 c.c. with alcohol. This slight modification not only removed precipitable material more thoroughly, but also enabled the end points in the titrations to be observed with greater certainty.

Table II. *Summary of analytical results.*

1. Green oats, tares and beans (calculated to dry matter basis).

	Bags 1, 2	Bags 3, 4, 5	Bag 6	Bags 7, 8
	%	%	%	%
Crude protein ...	9·69	8·46	10·69	8·10
Ether extract ...	4·09	3·85	4·16	3·09
N-free extractives	51·22	54·47	48·00	57·54
Crude fibre ...	26·19	25·69	29·66	25·24
Ash	8·81	7·53	7·49	6·03
True protein ...	7·55	5·85	8·11	5·61
"Amides" ...	2·14	2·61	2·58	2·49

2. Oats, tares and bean silage (calculated to dry matter basis).

	Bag 1	Bag 2	Bag 3	Bag 4	Bag 5	Bag 6	Bag 7	Bag 8
	%	%	%	%	%	%	%	%
Crude protein ...	10·28	9·86	9·30	9·60	9·90	10·87	9·45	8·61
Ether extract* ...	5·71	5·24	5·22	6·88	4·15	5·91	4·72	4·68
N-free extractives	46·57	47·88	49·19	45·97	46·70	46·80	51·21	52·38
Crude fibre ...	27·84	26·78	28·35	29·14	31·53	28·54	27·51	27·59
Ash	9·60	10·24	7·94	8·41	7·72	7·88	7·11	6·74
True protein ...	3·82	4·05	4·57	4·93	4·37	5·81	5·66	4·69
"Amides" ...	6·46	5·81	4·73	4·67	5·53	5·06	3·79	3·92

* Not taking into account volatile organic acids of silage.

Table III. *Analysis of silage extracts (calculated on basis of 100 gm. dry matter).*

	Bag 1	Bag 2	Bag 3	Bag 4	Bag 5	Bag 6	Bag 7	Bag 8
	%	%	%	%	%	%	%	%
Volatile organic acids* ...	2·18	2·30	2·95	2·74	2·98	2·46	1·32	2·32
Non-volatile organic acids†	4·22	4·45	3·92	4·04	3·74	5·46	1·76	3·38
Amino acids‡	4·79	4·59	3·12	3·29	4·12	4·02	2·20	2·64
Volatile bases‡	0·93	0·95	0·77	0·77	1·00	1·04	0·42	0·85

* Calculated as acetic acid (control figure for green crop was negligible).

† Calculated as lactic acid, after subtracting control figure for green crop.

‡ Calculated as crude protein.

Table IV. *Percentage gains or losses of constituents in bags 1 and 2, Silo 1 (containing very immature fodder).*

	Bag 1			Bag 2		
	Green crop	Silage	% increase or loss	Green crop	Silage	% increase or loss
	oz.	oz.		oz.	oz.	
Moist material ...	848·00	766·00	− 9·7	863·00	768·00	− 11·0
Dry matter* ...	187·00	168·80	− 9·7	190·30	167·90	− 11·8
Organic matter*	170·50	152·90	− 10·3	173·50	151·10	− 12·9
Crude protein ...	18·12	16·99	− 6·2	18·44	16·18	− 12·2
Ether extract* ...	7·65	12·94	+ 69·2	7·78	12·40	+ 59·4
N-free extractives	95·78	76·98	− 19·6	97·47	78·57	− 19·4
Crude fibre ...	48·98	46·02	− 6·1	49·84	43·95	− 11·8
Ash	16·47	15·87	− 3·6	16·77	16·80	+ 0·2
True protein ...	14·12	6·31	− 55·3	14·37	6·65	− 53·7
"Amides" ...	4·00	10·68	+167·0	4·07	9·53	+134·1

* Allowance made for silage volatile organic acids as acetic acid. Amount of silage dry matter calculated as residue after drying at 100° C.: 165·3 oz. (bag 1), 164·1 oz. (bag 2).

Table V. *Percentage gains or losses of constituents in bags 3 and 4, Silo 2 (containing fodder cut in early maturity).*

	Bag 3			Bag 4		
	Green crop	Silage	% increase or loss	Green crop	Silage	% increase or loss
	oz.	oz.		oz.	oz.	
Moist material ...	710·00	661·00	− 7·0	637·00	601·00	− 5·7
Dry matter* ...	187·70	170·80	− 9·0	168·40	153·90	− 8·6
Organic matter*	173·60	157·60	− 9·2	155·70	141·30	− 9·3
Crude protein ...	15·88	15·43	− 2·8	14·25	14·38	+ 0·9
Ether extract* ...	7·23	13·56	+87·5	6·48	14·41	+122·4
N-free extractives	102·24	81·61	− 20·1	91·73	68·86	− 24·9
Crude fibre ...	48·22	47·03	− 2·5	43·26	43·65	+ 0·9
Ash	14·13	13·17	− 6·8	12·68	12·60	− 0·6
True protein ...	10·98	7·58	− 31·0	9·85	7·38	− 25·1
"Amides" ...	4·90	7·85	+ 60·0	4·40	7·00	+ 59·1

* Allowance made for volatile organic acids in silage as acetic acid. Silage dry matter calculated as residue after drying at 100° C.: 165·9 oz. (bag 3), 149·8 oz. (bag 4).

Table VI. *Percentage gains or losses of constituents in bags 7 and 8, Silo 3 (containing fairly mature fodder).*

	Bag 7			Bag 8		
	Green crop	Silage	% increase or loss	Green crop	Silage	% increase or loss
	oz.	oz.		oz.	oz.	
Moist material ...	566·00	549·00	− 3·0	607·00	598·00	− 1·5
Dry matter* ...	174·00	154·30	− 11·3	186·70	175·90	− 5·8
Organic matter*	163·50	143·50	− 12·2	175·40	164·30	− 6·3
Crude protein ...	14·09	14·38	+ 2·0	15·12	14·80	− 2·1
Ether extract* ...	5·38	9·29	+72·7	5·77	12·04	+108·7
N-free extractives	100·12	77·94	− 22·2	107·43	90·04	− 16·2
Crude fibre ...	43·92	41·87	− 4·7	47·12	47·43	+ 0·6
Ash	10·49	10·82	+ 3·1	11·26	11·59	+ 2·9
True protein ...	9·76	8·61	− 11·8	10·47	8·06	− 23·0
"Amides" ...	4·33	5·77	+33·3	4·65	6·74	+ 44·9

* Allowance made for volatile organic acids in silage as acetic acid. Silage dry matter calculated as residue after drying at 100° C.: 152·2 oz. (bag 7), 171·9 oz. (bag 8).

Table VII. *Percentage gains or losses of constituents in bag 5 (containing material cut in early maturity) and bag 6 (containing material cut in medium maturity). Commercial silo.*

	Bag 5			Bag 6		
	Green crop	Silage	% increase or loss	Green crop	Silage	% increase or loss
	oz.	oz.		oz.	oz.	
Moist material ...	671·00	503·00	− 25·0	782·00	659·00	− 15·7
Dry matter* ...	177·40	143·40	− 19·2	181·80	168·40	− 7·4
Organic matter*	164·00	132·60	− 19·1	168·20	155·50	− 7·6
Crude protein ...	15·01	13·78	− 8·2	19·43	17·87	− 8·0
Ether extract* ...	6·83	9·98	+ 46·1	7·56	13·72	+ 81·5
N-free extractives	96·63	65·00	− 32·7	87·27	76·94	− 11·8
Crude fibre ...	45·57	43·89	− 3·7	53·92	46·92	− 13·0
Ash	13·36	10·75	− 19·5	13·62	12·95	− 4·9
True protein ...	10·38	6·08	− 41·4	14·74	9·55	− 35·2
"Amides" ...	4·63	7·70	+ 66·3	4·69	8·32	+ 77·4

* Allowance made for silage volatile organic acids as acetic acid. Silage dry matter calculated as residue after drying at 100° C.: 139·2 oz. (bag 5), 164·4 oz. (bag 6).

DISCUSSION OF ANALYTICAL RESULTS.

In examining the above tables, it will be seen that many of the figures raise points which have been discussed in detail in a previous communication. The present discussion will be limited to those results which bear directly on the special object of the investigation in hand, namely, the effect of the stage of maturity of the crop on the course of the changes which occur in the silo.

The results for the silage samples in bags 1, 2, 3, 4 and 8 may be taken together for purposes of comparison, since all these samples had been made in the small silos. The results for bags 5 and 6, which had been placed in the commercial silo, are not strictly comparable with the aforementioned, and will be discussed separately. Bag 7 silage presents certain abnormal features, owing to slight spoiling by mould.

Losses of juice and dry matter.

Maturity of crop ...	Very immature		Early maturity		Fairly mature
No. of bag	1	2	3	4	8
Loss of juice (oz.) ...	− 63·8	− 72·6	− 32·1	− 21·5	+ 1·8
% loss of dry matter	9·7	11·8	9·0	8·6	5·8

It will be seen that the percentage loss of dry matter during ensilage decreased as the maturity of the crop increased.

The loss in bag 8 is the lowest which has been recorded in this series of investigations, and as no loss owing to drainage occurred in this instance, the result indicates that the loss due to fermentation alone is

approximately 6 per cent. of the dry matter with fodder at this stage of maturity.

The results confirm the earlier findings of the writers[2] that the amount of drainage and the loss of dry matter are highest when immature crops are ensiled, and that the production of the "acid brown" type of silage is associated with the lowest losses.

The slight mould development in bag 7, which was filled with the same type of fodder as bag 8, led to a relatively high loss of dry matter, namely, 11·3 per cent.

Changes in the nitrogenous constituents.

The losses of nitrogenous substances were only appreciable in bags 1 and 2, the reason for this being found in the greater amount of drainage from the immature material in these bags. This leads to substantial losses of the soluble nitrogenous bodies constituting the "amide" fraction of the silage.

The results show that the hydrolytic changes affecting the protein constituent of the crop are most marked in the ensiling of the immature crop, the effect diminishing as the ensiled crop became more mature. In bags 1 and 2, more than 50 per cent. of the true protein of the green crop was hydrolysed during the ensilage process, this resulting in a very large increase in the amount of "amides" (roughly 150 per cent.). In the case of the "brown acidic" silage, however, only about 23 per cent. of the true protein was affected by this change, the resulting percentage increase of "amides" being as low as 45 per cent.

It may be concluded then that conditions of immaturity are favourable to the extensive splitting up of the protein of the crop into amino acids.

Nitrogen-free extractives and ether extract.

If fermentative activity in the silo be measured by the amount of breakdown of carbohydrates, then there is little in this respect to distinguish the ensilage processes with the crop at the three different stages of maturity. If anything, this type of activity was lowest with the most mature fodder (bag 8), since only 16 per cent. of the carbohydrates was destroyed, as against roughly 20 per cent. in the case of the immature fodder (bags 1 and 2).

The resulting increases of ether soluble constituents due to development of organic acids were not correlated with the amounts of carbohydrate fermented. This is again mainly due to the disturbing effect of

drainage, the effect, as would be anticipated, being most marked in bags 1 and 2. In the latter bags a 20 per cent. destruction of carbohydrates only resulted in a 60–70 per cent. increase of ether extract, a substantial proportion of the organic acids having been lost in the drainage. In bag 8, however, where no drainage losses occurred, a 16 per cent. loss of carbohydrates resulted in the ether extract being more than doubled.

The losses of organic acids are highest therefore during the ensilage of immature crops.

The titration of the silage extracts showed that all the samples contained a bigger proportion of non-volatile organic acids than volatile. The average ratio of non-volatile to volatile acids for bags 1, 2, 3, 4, 5, 6 and 8 was 1·63 : 1. Dox and Neidig(3) have demonstrated that this feature is characteristic of good silage and by direct determination of lactic, acetic and propionic acids in maize silage obtained a corresponding ratio of 1·34 : 1.

That the ratio alone is not a criterion of the quality of silage is shown by the results of bag 7 silage. In this case the value of the ratio was 1·33 : 1, in spite of the fact that the amounts of organic acids were abnormally low as a result of mould activity. The only safe criterion of quality involves a consideration of three factors: (1) Ratio of non-volatile to volatile organic acids. (2) Amounts of organic acids. (3) Type of volatile organic acid.

Crude fibre changes.

In previous communications(2) evidence has been brought forward showing that the cellulose of the oat and tare crop does not remain unaffected during ensilage, but that a proportion of the fibre undergoes breakdown, as a result of bacterial activity, with the probable formation of nitrogen-free extractives and organic acids. In addition, the residual fibre has been shown to possess greater digestibility than the original fibre of the green crop(6).

The evidence of the present investigation indicates that these changes may be controlled by particular conditions, one of these being the stage of maturity of the crop. It seems probable, as would be anticipated, that the cellulose of the succulent immature crop is much more readily affected by bacterial activity in the silo than is the more lignified cellulose of the maturer crop. In bags 1 and 2, for instance, the disappearance of fibre was fairly considerable, whereas in bags 3, 4 and 8 there was little or no evidence of such changes.

This finding is in harmony with the behaviour of sunflower fodder in the silo, the woody fibre of this crop undergoing no diminution in amount during ensilage (5).

Changes occurring in commercial silo.

The conditions under which the material in bags 5 and 6 was converted into silage in the commercial silo differed essentially in several respects from those obtaining in the small silos. The temperatures of fermentation were maintained at a higher level and the bags were subjected to greater pressures than could be attained in a small silo.

It is not surprising therefore that bag 5, which was in the lower half of the silo and which remained there some months after the removal of the other bags, should have suffered a very large loss of dry matter (19 per cent.). A considerable amount of juice was expressed from the material (approx. 134 oz.) and the effect of drainage is further reflected in the losses of nitrogenous constituents, organic acids and inorganic salts.

The low loss of dry matter (7·4 per cent.) from bag 6, which was placed in the upper half of the silo, was somewhat surprising in view of the large loss of moisture from this bag (about 110 oz.). This bag, however, was filled after a wet period and the material was very wet with superficial moisture, which presumably must have drained off quickly without carrying with it any appreciable amounts of constituents in solution.

The loss of crude fibre from this bag was considerable and suggests that the temperature of fermentation is another factor which controls the bacterial changes affecting the cellulose constituent.

Summary of results obtained with green "fruity" silage and "acid brown" silage.

The conditions controlling the ensilage of oat and tare crops are now understood with sufficient precision to enable the type of silage to be predicted at the time of filling the silo. For this reason it is safe to prophesy that the very undesirable "sour" silage so frequently obtained in the early days will quickly disappear altogether and the two kinds of silage which will commonly be made will be the green "fruity" and the "acid brown" types. It is therefore of interest to summarise the results which have been obtained in experiments dealing with these two kinds of silage.

Percentage gains or losses of constituents during ensilage.

	Green "fruity" silage (mean of 8 trials) % gain or loss	"Acid brown" silage (mean of 3 trials) % gain or loss
Dry matter ...	−11·2	− 7·7
Crude protein ...	− 8·2	0·0
Ether extract ...	+52·4	+45·0
N-free extractives	−19·1	−14·7
Crude fibre ...	− 5·5	− 6·0
Ash	− 9·2	0·0
True protein ...	−41·0	−28·4
"Amides" ...	+85·3	+96·0

The above summary brings out very clearly the essential differences between the chemical processes by which the two kinds of silage are produced. The loss of dry matter is greater in making the green "fruity" silage than that which is associated with the production of the "acid brown" type. This difference is largely a result of the more copious drainage which accompanies the ensiling of the less mature and more succulent crop and further evidence of this is afforded by a study of the losses of crude protein and inorganic salts. Appreciable losses of these constituents occur in the production of green "fruity" silage, whilst with the "acid brown" variety the losses are nil. Conditions of immaturity appear to favour the splitting up of carbohydrates and true protein, and the figures for "amides" show that drainage losses must deprive the green "fruity" silage of substantial amounts of these soluble nitrogenous products.

It may further be urged that the cutting of the crop in early to medium maturity in order to make green "fruity" silage results in the ensiling of a smaller weight of forage per acre than would be dealt with if the crop were cut at a maturer stage for the production of "acid brown" silage. Against this, however, must be set the fact that green "fruity" silage is distinctly superior to the "acid brown" type in palatability, digestibility and nutritive value. Investigations carried out in this Institute have shown that the production starch equivalent of 100 lb. of the dry matter of green "fruity" oat and tare silage is 45·6[6], whereas the corresponding figure for "acid brown" oat and tare silage is only 33·4[7].

In conclusion, the writers would like to express their thanks to Mr V. Thurlbourn for considerable assistance in connection with the analytical work of the investigation.

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THE REQUIREMENTS OF THE PIG FOR "VITAMIN A" AND "VITAMIN C."

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MOST of our information on the subject of vitamins has been derived from experiments on small animals fed on synthetic diets. The direct application of the results of such experiments to the feeding of farm animals under practical conditions is not warranted. Animals differ in the degree of their susceptibility to disease caused by lack of vitamins, and laboratory diets, composed of foodstuffs subject to abnormal physical or chemical processes, such as heating under pressure, or prolonged extraction with fat solvents, have no parallel in animal husbandry.

The only feeding experiments, the results of which are of value as guidance to the stock feeder, are those conducted on farm animals with foodstuffs actually used in practice. Such experiments are of special value at the present time, when, owing to the wide interest taken in vitamins, conclusions drawn from research work on this aspect of nutrition receive much attention from the practical agriculturist.

The farm animal which might be expected to be most likely to suffer from vitamin deficiency is the pig. It is liable to be kept in continuous confinement through the whole period of its life, and to receive a monotonous ration, which may consist largely of commercial by-products poor in one or more of the vitamins. Further, of all farm animals it is probably the one that suffers most from nutritional disorders, with symptoms resembling those usually associated with vitamin deficiency.

Of the three vitamins A, B and C, vitamin B, which is contained in grains, grain offal, and most of the natural foodstuffs fed to pigs, is almost certain to be present in sufficient amounts in any ordinary ration. The two likely to be lacking are vitamin A, or fat-soluble factor, and vitamin C, or anti-scorbutic factor.

The experiments recorded here were carried out to determine (1) whether the requirement of the pig for these two vitamins is such that

the animal is likely to suffer from deficiency of them when fed on rations of grain and grain offal; and (2) whether, on such rations, the addition of these vitamins leads to increased rates of growth.

Exp. 1. ANTI-SCORBUTIC FACTOR, OR VITAMIN C.

In this experiment two groups of pigs were fed on a basal ration with little or no anti-scorbutic. One group received the ration only. The other group received in addition a supply of anti-scorbutic vitamin C, in the form of lemon juice. The animals were weighed periodically, and notes taken of their condition.

Most of the work on scurvy has been done on guinea-pigs, which are highly susceptible to deficiency of anti-scorbutic. The requirements of the guinea-pig for anti-scorbutic, and the manifestation of scurvy in the guinea-pig are, therefore, well known. For that reason, a parallel experiment with guinea-pigs was run, to serve as a control, and as a basis of comparison of the results.

Experimental data.

Pigs. Eight pigs, between seven and eight weeks old, were arranged in two groups of four each, so that the average weights of the groups were approximately equal. The groups were comparable as to sex and litter; *i.e.* each pig in one group was balanced in the other group by a pig of the same sex, and out of the same litter.

Housing. The animals were housed in two pens, measuring each 12 feet by 6 feet, in an animal room with a cement floor. Boards were provided for sleeping on, but, to avoid the possibility of the animals eating anything in addition to the experimental ration, no litter or bedding of any kind was allowed.

Food. The ration consisted of:

			Proportions
Bran	...	...	100
Middlings	...	...	100
Crushed oats	...	...	100
Maize meal	...	...	100
Blood meal	...	...	25

To prevent complication of the results by deficiency of mineral matter or of fat-soluble A vitamin, the following was added to the ration:

Calcium phosphate	...	15 grams per pig per day.
Magnesium sulphate	...	2 ,, ,, ,,
Potassium carbonate	...	2 ,, ,, ,,
Ferric chloride	...	1·5 ,, ,, ,,
Cod-liver oil	...	10 c.c. ,, ,,

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The above ration is as poor in vitamin C as any ration likely to be fed to pigs. According to the Report of the Medical Research Council (1919), cereals (whole grain or bran) contain no anti-scorbutic, and as this vitamin is destroyed by heating or drying, it may be assumed that blood meal contains little or none.

One group of pigs—the experimental—was fed this ration, wholly, or almost wholly devoid of vitamin C. To the other group—the control —there was given in addition to the ration, fresh lemon juice to the amount of 1 c.c per kg. live weight per day. The juice was mixed with the food.

Water was always available and the animals were allowed to eat and drink according to their appetites.

Guinea-pig control.

Two groups of young guinea-pigs, three in each group, were kept in cages with wooden floors, in the same room as the pigs, and were fed on the above ration. One of the groups received the ration only; to the food of the other there was added 5 c.c. lemon juice per kg. body weight per day, *i.e.* five times as much in proportion to their weight as the control pig group.

Results.

The following table shows the rate of increase of weight in the pigs during 111 days, after which the experiment was stopped.

Table 1. *Weight of Pigs in kilograms.*

No. of pig	15. vi. 21	15. vii. 21	14. viii. 21	13. ix. 21	4. x. 21	Average gain over the period	
3	11·3	17·1	26·9	43·0	56·6	0·41	10 c.c. lemon juice per pig per day
55	11·4	16·9	29·1	45·8	61·2	0·45	
57	13·2	21·0	31·9	51·0	67·1	0·49	
64	12·5	16·2	23·2	37·0	50·6	0·34	
2	9·5	14·5	25·8	41·2	58·3	0·44	No anti-scorbutic
56	14·1	21·6	35·5	53·9	71·6	0·52	
58	14·3	21·5	31·6	48·8	66·7	0·47	
65	10·3	16·4	26·0	43·4	58·1	0·43	

The average increase in weight per day in the control group was 0·421 kg., and in the experimental group 0·465 kg. The average gain in the former group was decreased by pig No. 64, which was from the same litter as No. 65 of the experimental group. This animal grew less rapidly than the others in the early part of the experiments. Even omitting this animal, the rate of gain in the experimental group is slightly greater than in the control group.

On the 111th day, when the experiment was stopped, all the animals appeared in perfect health. No difference could be detected in the condition of the coats, the activity. or the "thrifty" appearance of the two groups.

Guinea-pig control experiment.

In the group receiving no anti-scorbutic the well-known signs of scurvy appeared in all the animals during the third week. One died on the 21st day, one on the 22nd, and the third was losing weight. The post-mortem examinations showed the well-known signs associated with scurvy.

In the group receiving the lemon juice, signs of scurvy appeared in one of the animals during the third week. It died on the 23rd day. The other two showed no signs except that the rate of increase of weight slowed down.

As the object of this control experiment had been accomplished as soon as it was demonstrated that the diet given to the pigs was really deficient in anti-scorbutic, a double amount of lemon juice was given to all the surviving guinea-pigs from the 24th day onwards. The food, with the increased allowance of lemon juice, was continued until the 45th day from the beginning of the experiment. All the animals were then evidently in good health and showing normal gains in weight.

The results of these experiments show that the pig can be maintained in health for at least 111 days, on a ration that allows the development of definite signs of scurvy in the guinea-pig within 20 days.

Exp. 2. FAT-SOLUBLE A OR VITAMIN A.

The experiment recorded here deals with the influence on the rate of growth and general health of the addition of cod-liver oil to a ration poor in vitamin A.

Experimental data.

The conditions of the experiment were the same as in Exp. 1, with which it ran concurrently.

The basal ration consisted of:

				Proportions
Bran ...	...	...	...	100
Crushed oats ...		...	...	100
Blood meal ...	...	...	...	8

Anti-scorbutic was supplied in the form of 20 c.c. fresh swede juice per pig per day, mixed with the food.

The following salts provided additional mineral matter and neutralised the excessive acidity of the ration.

				Amounts given
Calcium phosphate	...	8 parts	}	20 grams of
Magnesium sulphate	...	1 part	}	mixture per
Potassium carbonate	...	1 ,,	}	pig per day
Ferric chloride	...	0·8 ,,	}	
Calcium carbonate...	...	7·5 grams	}	per kilo of ration
NaOH 10 %	...	30 c.c.	}	

It is unlikely that any ration fed to pigs in practice could contain less fat-soluble A than is found in the above. According to the Report of the Medical Research Council the cereals (grain or bran) contain none. The blood meal used contained only 0·24 per cent. of fat, and whatever vitamin was originally present, would be partially, if not wholly, destroyed in the process of manufacture. The blood meal, which was added to ensure that the protein would be sufficient in quantity and quality to support growth in the early stages, when a narrow albuminoid ratio is required, was gradually decreased, and was omitted altogether at the end of the second month.

To the food of one group there was added 10 c.c. of cod-liver oil per pig per day, and to that of the other an equal amount of linseed oil. Cod-liver oil is abundantly rich in fat-soluble A. Linseed oil is poor in this vitamin. According to the Report of the Medical Research Council it contains none.

Results.

The following table gives the individual gains in weight over a period of 110 days.

Table II. *Weight in kilograms.*

No. of pig	15. vi. 21	15. vii. 21	14. viii. 21	13. ix. 21	3. x. 2	Total gain over whole period	
63	10·1	16·3	25·8	38·5	49·5	39·4 }	
107	10·1	13·0	19·8	30·5	38·5	28·4 }	Linseed oil
108	9·7	13·0	20·2	30·3	37·8	28·1 }	
110	10·6	14·3	22·7	37·6	46·3	35·7 }	
61	10·9	16·9	27·3	42·3	53·0	42·1 }	
106	9·4	10·1	15·7	27·5	36·2	26·8 }	Cod-liver
109	9·7	11·6	16·5	28·8	38·2	28·5 }	oil
112	10·4	12·2	19·8	32·8	43·1	32·7 }	

For the first few weeks none of the animals throve, probably owing to the large amount of fibre in the food. During this period the "linseed oil" group gained in weight more rapidly than the "cod-liver oil" group. Towards the end of the experiment the reverse was the case. For the whole 110 days there was little difference in the rate of growth, as indicated by weight, or as could be judged by the appearance. The

linseed oil group gained, on an average, 0·299 of a kilo per day, and the cod-liver oil group 0·296. All the animals appeared normal, and in good condition, at the end of the period.

Exp. 3. Addition of vitamins A and C to an ordinary cooked ration.

In this experiment an attempt was made to determine whether the addition of vitamin A and C to a ration commonly fed to pigs during the fattening period would result in increased rates of gain in weight. Foodstuffs commonly used were chosen to form the ration. As vitamin C and to a less extent A are said to be destroyed in cooking in the presence of air, and as it is a common custom, especially when feeding potatoes, to boil foodstuffs for pigs, the basal ration was subjected to prolonged heating. The cooked basal ration was fed to one group, and to the other group vitamins were added, and the influence on rate of growth and on condition was noted.

Experimental data.

Animals and Housing. Nine pigs, four months old, out of the same litter were divided into three groups of three each, and housed separately in pens. The conditions with regard to housing were comparable in the three groups. As a control three pigs of the same age and breed, but of a different litter, were allowed to run on pasture.

Food. The basal ration consisted of:

			Proportions
Potatoes	...	...	600
Middlings	...	...	200
Locust bean meal	...	...	150
White fish meal	...	...	100

During the experimental period the "nutritive ratio" was gradually reduced from 1 : 4·5 to 1 : 6·4 by the addition of maize meal.

The ration was brought to boiling-point and kept boiling for an hour. It was then left in the boiler overnight. The fish meal had already been subjected to a high temperature for about 10 hours during the process of manufacture.

The following additions were made to the ration. The amounts stated were for the three pigs of the group. The cut grass was given in a separate trough. The additions were mixed with the food.

Group I	Group II	Group III	Group IV
60 c.c. cod-liver oil 400 grams fresh grated swede turnip	1300 grams fresh cut pasture grass	150 grams oatmeal	Liberty to graze

In Group I the cod-liver oil gave an abundant supply of vitamin A, and the swede of vitamin C. In Group II the fresh pasture grass gave a rich supply of A, B and C. In Group III the oatmeal, which contains no vitamin, was added to balance the energy value of the additions to Groups I and II.

A box containing a mixture of chalk, coal and wood ashes, and sulphur was provided for each pen, and water was always available.

The amount of food given was regulated by the appetite, which was fairly uniform throughout the groups. All the animals had the same amount of food per day.

Results.

The following table shows the average gains in weight during the feeding period of 64 days.

Table III. *Average weights in kilograms.*

	2. ix. 21	5. xi. 21	Gain
Cod-liver oil and swede	40·7	90·2	49·5
Grass	40·4	87·6	47·2
Oatmeal	39·7	88·5	48·8
	5. ix. 21	5. xi. 21	
Grazing	40·6	88·6	48·0

The small differences in gain are insignificant, especially as the caloric value of the additions to the basal ration were only roughly adjusted, and Group II did not always clean up all the green stuff given. All the animals were in excellent condition on the 64th day, when this part of the experiment finished.

Two of the pigs in the oatmeal group were continued on the ration for a further period of 64 days, to ascertain whether any sign of malnutrition would appear with prolonged feeding on the cooked ration. At the end of the period the animals appeared in perfect condition, and post-mortem examination showed no abnormality of bones or other tissues.

The following table shows the gains in weight of these two animals over the whole period of 127 days.

Table IV.

Weight in kilograms			Average gain
2. ix. 20	5. xi. 20	8. i. 21	per day
44·4	95·0	140	0·75 kg.
36·3	86·7	126	0·71 ,,

It seems doubtful therefore, whether, during the period of fattening, the addition of either vitamin A or vitamin C to ordinary rations fed to pigs leads to any beneficial results.

DISCUSSION OF RESULTS.

Sherman(1921) states that scurvy occurs in the pig, but gives neither data, nor reference to data, in support of the statement. Plimmer (1920) describes as scurvy a condition of malnutrition which occurred in four months old pigs fed on meal, sharps and turnips. He considered that the disease had been cured by stopping the cooking of the food. The condition noted was most probably due to deficiency of calcium. Orr and Husband (1922) found that a four months' old pig requires from 10 to 12 grams of calcium (as CaO) per day. This is merely a confirmation of older work. Kellner(1907) recommended that growing pigs should have 5 to 12 grams of chalk per day added to their ration to secure a sufficient supply of calcium. A reference to the tables of Forbes(1913) or König(1919) shows that the largest ration of turnips and cereal products that a pig could consume would contain a mere fraction of the amounts shown to be necessary. The improvement in the condition of the animals in question was therefore most probably due not to any change in the ration, but to the fact that they were turned out to pasture, and allowed to graze and root. The mineral matter of the pasture tends to correct the natural deficiency of cereals, especially with regard to their deficiency in calcium.

The results of Exp. 1, quoted above, indicate that the pig is little susceptible to scurvy. In this it resembles the rat, which can make a complete life-cycle on a diet with no anti-scorbutic, without showing any of the signs of scurvy; or the prairie dog, which McCollum(1920) has shown to thrive for six months on a diet similarly deficient.

The results of Exp. 3 show that the mere cooking of a ration is not in itself sufficient to produce scurvy in pigs, even assuming that it is possible to produce that condition in pigs at all.

Some observations on the influence of fat-soluble A on the rate of growth of pigs have been made by Zilva, Golding, Drummond and Coward(1921) in the course of an investigation the primary object of which was to determine whether pigs on a diet deficient in this accessory factor would develop rickets. They found that "no definite rickets was produced in sucking-pigs fed from birth on a diet rigorously restricted in the fat-soluble factor." With regard to the rate of growth, they conclude that "the addition of the fat-soluble factor in the form of

cream, cod-liver oil, or lucerne to a deficient diet stimulated growth in pigs declining in weight."

The experiment was done with four young pigs which were put on the experimental ration when only four days old. Two were given a diet roughly similar in composition to sow's milk. It contained fresh cow's milk, crude caseinogen, cream and a salt mixture. Other two were given a diet similar in composition except that it contained no fat-soluble A. The pigs on the fat-soluble A-free diet did badly in the beginning of the experiment, eating less and putting on less weight. One died, and to save the other cream was added to its ration for 13 days. During that period appetite and growth were improved, and at the end of it the cream was withdrawn. Thereafter this pig "in spite of the restricted diet kept on growing after the resumption of growth produced by the adminis- tration of the cream, even better than the animals in the control group." The experiment began on August 17th. On November 26th the weight of the animal on the diet poor in the fat-soluble A despite the initial check was 72 lb.; the weights of the two on the diet rich in fat-soluble A were $65\frac{1}{4}$ and $72\frac{1}{4}$ lb. respectively.

This experiment was much better controlled with regard to the amount of fat-soluble A present than those recorded above. The con- stituents of the diet were freed from this factor by chemical or physical processes. Any fat-soluble A the animal could have got must have been given in the cream which was fed for a few days at the beginning of the experiment, and any that may have been present in the unpurified caseinogen which, for the greater part of the experiment, was given instead of caseinogen which had been treated to remove any trace of vitamin A. In view of the result, viz. that the pig on the diet so rigorously restricted in fat-soluble A grew, on the whole, better than its neighbours which were on the diet rich in this factor, the conclusion of the writers that "the requirement of the pig for the fat-soluble factor seems of a low order" would appear to be completely justified.

In the other experiments of these writers, where the addition of lucerne or cod-liver oil produced improvements in growth, the animals were on a ration of "toppings" and whey. This ration is markedly deficient in mineral matter and especially in calcium. Sow's milk con- tains in percentages: protein, 7·2; CaO, 0·41; P_2O_5, 0·35 (König). In toppings, *i.e.* wheat offal, the percentages are: protein, 17·8; CaO, 0·08; P_2O_5, 21·1. The ratio of calcium to protein in sow's milk is about 1 : 17. In toppings it is only about 1 : 200. In whey the percentage of calcium is variable, but always less than in cow's milk, where it varies

from 0·13 to 0·17. The animals must, therefore, have been suffering from a marked deficiency of calcium, and a great excess of phosphorus. "Lucerne," or Alfalfa hay, contains in percentages: protein, 14·9; CaO, 1·95; P_2O_5, 0·54. The lucerne would therefore tend to correct both the deficiency of calcium and the wrong ratio of calcium to phosphorus. It would also supply iron. Alfalfa hay contains 0·169 per cent. Fe_2O_3, whole wheat only 0·02 per cent., and whey certainly not more than whole milk, which contains only 0·0001 to 0·0002 per cent. (Hess and others, 1920). In view of the results of Elliot, Crichton and Orr (1922) on the relative influence of mineral deficiency and vitamin A deficiency on the growth of young pigs, the influence of the lucerne can, most probably, be taken as due to the mineral content rather than to its vitamin content.

In the case of another sow on the same mineral deficient ration, the addition of crude cod-liver oil was followed by increased rate of growth. The results of experiments by Husband, Godden and Richards(1923), carried out at this Institute during the past two years, show that oil *per se* has an influence in increasing the percentage of calcium absorbed from the intestine. It is probable therefore that the beneficial results noted were not entirely due to vitamin A. In any case the influence of the oil was apparently not sufficient to compensate for the deficiency of the ration, because the young pigs from this sow which, during pregnancy, was again on the "whey and toppings" ration, were born defective. The young pigs showed oedema, with ascites, hydropericardium and hydrocele-phenomena somewhat similar to that found by McGowan and Crichton (1923) in the young of pigs sucking sows on an iron-deficient diet.

It is noteworthy that it was the sow that, for a period, received the cod-liver oil, that had the defective litter, and that the sow which, for the same period, had lucerne, rich in calcium and iron, had a normal, healthy, litter. Cod-liver oil is the richest known source of fat-soluble A. According to Zilva and Miura(1921) it contains several hundred times as much as butter. The beneficial effects of lucerne are therefore to be ascribed to the minerals present, rather than to fat-soluble A.

A most interesting series of experiments which throw light on the requirements of the pig for vitamins, although they were not carried out for that purpose, are recorded by McCollum and Hoagland (1913). They were studying the endogenous metabolism of the pig, and, for experimental purposes, fed young pigs on a diet with no protein. The ration in all these experiments consisted for a time of nothing but starch and

salts, with some agar-agar, an indigestible carbohydrate, to secure evacuation of the bowels. In one of the experiments the animal continued for the whole experimental period of 73 days on a diet of starch and salts. No mention is made of any signs of vitamin deficiency, and it is unlikely that experienced workers like McCollum and Hoagland would have continued to carry out physiological experiments with an animal in a pathological condition. It may be assumed therefore that the gross signs of disease associated with deficiency of anti-scorbutic, or fat-soluble A factor, did not appear. Signs of vitamin deficiency tend to appear earlier in cases where the ration is badly balanced, and especially when there is an excess of carbohydrate (McCarrison, 1921). In this case the energising part of the ration consists of nothing but starch—a pure carbohydrate. From the result of this experiment, together with that of Zilva and others, in which the growing pigs on the diet rigorously restricted in the fat-soluble factor made better gains in weight than the controls on the vitamin A rich diet, it would appear that the requirement of the pig for vitamin A or C must be remarkably low, provided the mineral matter of the diet is adjusted to the requirements of the animal.

Crowther (1922) carried out a series of feeding experiments with growing pigs. He used cereal meals as the basal ration. The cereals are lacking in anti-scorbutic and contain very little fat-soluble factor (Med. Res. Report, *l.c.*). He found no definite improvement in rate of growth on the addition of either green food, which supplies both vitamin A and C, or of cod-liver oil.

In a more recent practical feeding experiment carried out by White and Roberts (1923) the results obtained by Crowther were confirmed. The experiment ran for 112 days. During that period the animals that were fed on the ration with the low content of anti-scorbutic factor, and fat-soluble factor, and were confined in pens, made an average gain in weight of 1·37 lb. per day compared with 1·35 lb. in the control group which had green food *ad lib.* in addition to the basal ration. There was no sign of nutritional disease in the former group. They "looked particularly well right through the experiment." These workers conclude that "there must be less danger of pigs suffering from lack of 'vitamins' than is often suggested."

In the experiments carried out by the writers no definite beneficial effect seemed to accompany the addition of vitamin C or the fat-soluble factor to a ration purposely arranged to be as deficient in these as any ration likely to be fed in practice.

It may be concluded that the requirement of the pig for these two

vitamins is so low that, during the usual fattening period, *i.e.* between weaning and slaughter, there is little likelihood of pigs suffering from vitamin deficiency under practical feeding conditions.

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A NOTE ON SOIL SHRINKAGE.

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In a recent paper by W. B. Haines[1] a study has been made of the shrinkage a soil undergoes during drying. It was shown that the shrinkage takes place in two stages in each of which there is a linear relationship to the moisture content. If the volume shrinkage during drying is plotted against the water content (calculated on a volume basis) the curve obtained consists of two straight lines cutting one another sharply. The first portion of the curve is inclined to the horizontal axis at an angle of 45° indicating that the volume shrinkage is exactly equal to the volume of water lost. The final portion of the curve, near the "dry" end and representing what Haines calls the residual shrinkage, is also straight (or nearly so) and is inclined to the horizontal axis at a much smaller angle than 45° indicating that the volume shrinkage in this region of the curve is much less than the volume of water lost. This portion represents the shrinkage that occurs when the drying process has been carried so far that the pore space is no longer full of water but contains air that has replaced some of the lost water.

Many of the quantitative data adduced by Haines are in accord with some of the writer's experimental results on the evaporation of water from soil[2] and a comparison of the two sets of data yields some interesting conclusions. At the same time there are differences of interpretation that are difficult to reconcile with the experimental facts and in the present note a brief examination of these points of agreement and difference will be made.

The two sets of data are not strictly and quantitatively comparable as different soils were dealt with in the two cases. Kaolin, however, is probably a sufficiently uniform product to justify a strict comparison. With this material the break in the shrinkage curve occurred at 24·8 per cent. water content (calculated on a dry weight basis). In this case no further shrinkage occurred, *i.e.* with kaolin there is no residual

[1] This *Journal*, 1923, **13**, 296.
[2] *Ibid.* 121; *Roy. Soc. Proc.* 1923, **103** A, 139, 664.

shrinkage, the shrinkage being wholly of what may be called the normal type, since the curve is inclined to the axis at 45°. At this point on the curve the pore spaces begin to empty, air to some extent replacing the evaporated water. The value obtained by the writer for the critical moisture content (*i.e.* the moisture content at which the rate of evaporation begins to fall off from constancy) of kaolin was 13·5 per cent. From this it would appear that the whole of the water, loss of which produces normal shrinkage, is water the evaporation of which is expressed by part of the horizontal portion of the rate of evaporation curve. The water content at which the shrinkage changes from normal to residual seems to be much greater than that at which the rate of evaporation begins to fall off from constancy. The residual shrinkage curve would correspond therefore with a portion of the horizontal and the whole of the sloping portions of the writer's evaporation curves for kaolin, sand, silty soil, ball clay, etc. This is significant because the writer has shown that any imbibitional or "gel" water present is given off along the horizontal portion of the rate curve, except in those cases (*e.g.* Rothamsted subsoil) where the colloid content, and consequent imbibition, is too great. In such cases the evaporation of imbibitional water is continued beyond, *i.e.* below, the critical moisture content producing curvature in the evaporation curve. This seems to indicate that normal shrinkage is due to the loss of both imbibitional and interstitial water when these are present together.

This conclusion is strengthened by a further comparison of the data. The critical moisture contents, corrected for curvature, of the Rothamsted subsoil (clay fraction = 55·4 per cent.) and the fractionated subsoil (clay content unknown but certainly very much more than 55·4 per cent.— probably 80 or 90 per cent.) are 21 and 35 per cent. respectively, while the moisture contents at which the curvature ceases, *i.e.* at which the whole of the imbibitional water has been given off, are 7·5 and 18·5 per cent. respectively. The soils used by Haines that are most comparable to these were a clay separate (clay fraction = 90·5 per cent.), a Sudan clay (clay fraction = 42·3 per cent.) and a Durham clay subsoil (clay fraction = 33·8 per cent.). The moisture contents (calculated on a dry weight basis) at which the normal shrinkage gave place to residual shrinkage are, according to Haines, 16·7, 13·0 and 15·2 per cent. respectively. While the comparison is not strict owing to the considerable differences between the two sets of samples, there can be little doubt that these values are *below*, and perhaps well below, the critical moisture contents. It is highly probable that these soils would exhibit curvature

in their evaporation curves showing that evaporation of imbibitional water is continued below the critical moisture content: since the normal shrinkage is also probably continued below this point the presumption is strong that both imbibitional and interstitial water are equally responsible for the normal shrinkage.

Haines[1] suggests that the total amount of shrinkage undergone by a soil when dried from its point of maximum plasticity to the beginning of residual shrinkage is linked with the clay content, in which case imbibitional water should be jointly responsible. At the same time Haines maintains that the residual shrinkage depends on the organic content which he supposes forms a film or colloidal coating or pad round the soil particles. "*Owing to the highly colloidal nature of these pads they would not part with their water until the later stages of the drying of the soil*[2], that is to say, their part in the shrinkage would be played after air began to displace water in the soil pores. This is the region of residual shrinkage." The writer's work has shown that "colloid," *i.e.* imbibitional, water evaporates with great ease and comes off before the last several per cent. of interstitial or capillary water: this applies as much to organic colloidal matter as to mineral colloidal matter or clay[3]. In view of these considerations it is difficult to accept the theory that residual shrinkage is due to evaporation of "colloid" water from films of organic matter that surround the soil particles. The fact that residual shrinkage could not be induced in kaolin by adding up to 1·5 per cent. of gelatin is significant. Haines' suggested explanation of this that "it must be supposed that the gelatin remains distributed throughout the water without any tendency to form films round the kaolin particles" is also difficult to accept[4]. At the point of inflexion the water present would appear to have a concentration of gelatin of nearly 4 per cent. which would form a gel at ordinary temperatures. Almost the whole of this gel would be in the form of annular "water" wedges between the kaolin particles, but, on general grounds, a concentration of gelatin with possibly the formation of a separate rigid gelatin phase at the water-kaolin interface might be expected to occur. If so, the actual film must be extremely thin, so that the mineral particles themselves must form a framework in which each

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[1] This *Journal*, *loc. cit.* 304.

[2] Italics are the writer's.

[3] E. A. Fisher, This *Journal*, *loc. cit.*; further details will be given in a later paper in this *Journal*.

[4] The following paragraph was rewritten and considerably extended in collaboration with Mr W. B. Haines, to whom the writer is glad to express indebtedness for much helpful criticism.

point of contact is rigid, bearing some resemblance to a glued joint. There cannot, of course, be any *swollen* gelatin in the film, as otherwise residual shrinkage would occur. The film must be supposed to consist rather of gelatin that has been denatured, *i.e.* removed from "solution" and as it were "precipitated" upon the kaolin surface, much as in the formation of a skin over the surface of a cup of cocoa and other surface films on colloidal solutions, such as those investigated by Ramsden[1], Metcalfe[2], Shorter[3] and others. Such an effect has been investigated by Zsigmondy[4] in the "protection" of gold sols by gelatin and it is on such action that J. Loeb[5] has recently formulated an extremely interesting theory of "protection" by colloids. It would be curious indeed if so essentially adhesive a material as gelatin did not form films round sand or other mineral particles. With coarse sand, which shows no tendency to cake on drying, the writer obtained a hard "brick" by moistening with a dilute gelatin solution, cooling and drying; it is difficult to believe that the strength of such bricks can be due only to the extremely thin films of dry gelatin interlacing through the spaces of the kaolin framework with no adhesion between the two systems. Such adhesion of thin precipitated films seems to the writer the obvious explanation of the considerable increase in pore space that occurred in these gelatin-kaolin systems. This, as Haines suggests, is largely due to the increased pore space at the time of mixing, but the point is that at low water contents this thin film of gelatin—the glued joints as one might almost call them—may be sufficiently strong to prevent that slipping of large numbers of kaolin particles over one another that would be necessitated by a reduction in pore space at these small water contents. Moreover, judging from the curves in Haines' paper (p. 307), there are distinct, though slight, indications of residual shrinkage. Thus the normal shrinkage ceased at about 50 per cent. water content, after which there appears to be a slight residual shrinkage of nearly 3 per cent., which however ceased entirely at a water content of 30-35 per cent. owing, if the above considerations are valid, to the increasing strength of the thin gelatin films. With no gelatin, on the other hand, normal shrinkage ceased suddenly at 24·8 per cent. water content after which there was no indication of any residual shrinkage.

On the other hand, residual shrinkage was induced in kaolin by generating silica gel on the surface of the particles. This certainly seems

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[1] *Roy. Soc. Proc.* 1903, **72**, 156; *Zeit. phys. Chem.* 1904, **47**, 342.

[2] *Zeit. phys. Chem.* 1905, **52**, 1.   [3] *Phil. Mag.* 1906, **11** [6], 317; 1909, **17** [6], 560.

[4] *Kolloidchemie* (Leipsic, 1918), 173, 358.   [5] *Journ. Gen. Physiol.* 1923, **5**, 479.

to point to a film or pad of some sort being necessary for residual shrinkage to occur but one must look elsewhere than to the typical "gel" or imbibitional water for the explanation. The non-imbibitional water of colloids (other than capillary water), such as water held in solid solution or chemically (such, for example, as the so-called water of hydration of many minerals, *e.g.* zeolites), is held somewhat tenaciously, but is far too small in amount (2 or 3 per cent.) to be a serious factor in the problem under consideration.

A provisional explanation can be suggested if it be assumed that the "pad" postulated by Haines is of lower mechanical strength than the soil particles themselves, so that it is less able to withstand forces tending to produce distortion. Residual shrinkage begins as soon as air begins to replace some of the lost water, that is as soon as water wedges with free menisci appear between the soil grains. When these menisci are less than $3\,\mu$ in diameter the wedges will be under a tension tending to pull the soil particles (already presumably in contact) closer together. This pull, small at first, increases rapidly as the water wedges by evaporation recede further and further into the interstices between the soil grains until, when the meniscus has a diameter of $2\cdot4\mu\mu$, the calculated pull may be as much as 1170 atmospheres. Forces of such magnitude may easily be sufficient to cause rupture of the pads—whether organic or mineral in nature—especially if the relatively dry soil mass is not packed in the closest possible manner. This is most likely to be the case with the peaty soil which had the biggest pore space (50·5 per cent.) as well as the largest residual shrinkage (18·2 per cent.) of all those examined. Kaolin when treated with silica gel not only gave a residual shrinkage of 3·2 per cent. but also an increase in pore space of about 3 per cent., while the total shrinkage has increased by only 1·7 per cent. The almost complete absence of residual shrinkage in the gelatin-treated kaolin (in spite of the large increase in pore space of from 37·8 per cent. to 48·5 per cent.) is probably due to the relatively enormous strength of thin films of gelatin: it is well known that under favourable conditions a good glued joint is stronger than the surrounding timber and the same may be true of kaolin.

A comparison of the experimental results of Haines and those of the writer enables one to define more closely than previous work allowed the significance to be attached to the critical moisture content[1] and to

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[1] It is perhaps unfortunate that the writer should have used this term in view of its earlier use in another connection by Cameron and Gallagher (*U.S. Dept. Agric., Bur. Soils,* Bul. 50, 1908). As used by the writer it means merely the moisture content at which the rate of evaporation begins to fall off from constancy.

explain the divergence between the first sloping portion of the evapora-
tion curve and the corresponding portion of the vapour pressure curve.
In kaolin for example, as has already been stated, normal shrinkage
ceases at a water content of 24·8 per cent. and this is also the point at
which air enters the pore space to replace water lost by evaporation.
At this point, therefore, annular water wedges with free menisci begin
to appear between the soil grains and evaporation proceeds from these
as well as from the relatively thick water films round the particles. As
evaporation proceeds from the water films and wedges near the surface
the lost water is largely replaced by water from the interior of the mass
moving outwards by capillarity through the interstices and through the
films. Evaporation therefore proceeds at a constant rate as no appreci-
able alteration in surface area occurs. At a lower water content, viz. at
the critical moisture content (13·5 per cent. in the case of kaolin), a point
is reached at which the water films have become so thin that water
can move through them only with great slowness; their vapour pressure
falls rapidly to such an extent that evaporation from them becomes too
slow for it to be a serious factor in the total rate at this stage. The
evaporation therefore takes place almost entirely from the water wedges
and as it proceeds the wedges recede progressively further into the
interstices between the soil grains; the menisci that constitute the
evaporating surface become progressively smaller in area long before they
begin seriously to diminish in vapour pressure and a fall in rate of evapo-
ration follows. It is unlikely that vapour pressure operates as a serious
factor in determining the shape of the evaporation curve until quite small
water contents are reached. Thus Thomas[1] showed that with a Greenville
clay loam containing 27·29 per cent. of particles smaller than 0·005 mm.
the vapour pressure was depressed by only 2 per cent. when the water
content was reduced to 9 per cent., while with a water content of 6 per
cent. the depression of the vapour pressure was only 5 per cent. With a
clay separate containing 44·8 per cent. of particles below 0·005 mm. and
63·2 per cent. of particles between 0·02 and 0·005 mm., the water contents
corresponding to 2 per cent. and 5 per cent. depressions of the vapour
pressure were a little less than 14 and 10 per cent. respectively. Reasons
are given in the writer's earlier paper[2] for supposing that the rate of
evaporation below the second break in the rate curve is independent of
the vapour pressure, being determined almost solely by the slowness of
movement of water within the soil mass. If these reasons are valid it
would appear that progressive lowering of vapour pressure as drying

[1] *Soil Sci.* 1921, **11**, 409.   [2] *Roy. Soc. Proc., loc. cit.*

proceeds has little, if any, influence in determining the *shape* of the evaporation curve although it may exert some slight effect on the slopes[1].

SUMMARY.

A recent paper by W. B. Haines on soil shrinkage is discussed and his explanation of residual shrinkage criticised in the light of earlier work by the writer on evaporation of water from soil. A modification of Haines' tentative explanation is suggested.

The experimental results of Haines have rendered it possible to define more clearly than previous work allowed the significance of the critical moisture content and the divergence between the first sloping portions of the rate of evaporation curves and the corresponding portions of the vapour pressure curves.

[1] Paragraph 1 of p. 129 of a former paper (This *Journal*, 1923, **13**, 121–43) should be re-interpreted in the light of these considerations.

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ON THE PERCHLORATE METHOD FOR THE ESTIMATION OF POTASSIUM IN SOILS, FERTILISERS, ETC.

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THE perchlorate method for the estimation of potassium in fertilisers, soil extracts, and plant ashes has been studied by Davis[1], working in these laboratories. He carried out a careful investigation into the sources of error of the method and claimed that when used according to the conditions worked out by him, the method was "more simple in manipulation and more uniform and exact in its results" than the older platinum chloride method. In general, this claim seems to be well founded, but from time to time cases have come to the author's notice, in which erratic results were obtained. Indeed, sporadic outbreaks of irresponsibility appear to be a feature of the method. In general, the position is summed up in the statement that the perchlorate method, while more convenient and less expensive than the platinum method, and usually at least equally accurate, is nevertheless liable occasionally to give faulty results erring on the side of highness.

The author's attention was forcibly directed to this question recently, owing to the sudden failure of the method in these laboratories, many cases of soil, fertiliser, and plant material analysis giving wildly discordant duplicates and impossibly high results. This was accompanied by an epidemic of deflagrations, which were liable to occur when alcohol was added to the residue after concentration of the perchloric acid to the fuming stage; these deflagrations were particularly liable to occur in soil and plant analyses, in which baryta had been added in order to remove sulphates. At the same time, great difficulty was experienced in carrying the concentration to the fuming stage, many times the usual amount of perchloric acid being needed, and even then the fuming was not usually as dense as it should be, but was accompanied by an unusually strong smell of "euchlorine." Attention was immediately directed to the purity of the perchloric acid in use. None of the impurities for the presence of which tests are provided in the "List of Reagents for

Analytical Purposes," issued by the Institute of Chemistry(2) could be detected. An investigation of the precipitates of supposed potassium perchlorate, the weights of which gave such impossibly high results, revealed the presence of barium in those precipitates obtained from soil and plant analyses and of a sodium salt and a chloride in the precipitates from analyses of potash manure salts. With all such precipitates it was also found that many successive washings with 95 per cent. alcohol (saturated at the laboratory temperature with potassium perchlorate) brought about successive reductions in weight, corresponding to the gradual removal of a barium or a sodium salt from the precipitate. For some time the cause of these abnormal occurrences was a matter of considerable mystification, until it occurred to the writer that an explanation was to be found in the possible contamination of the perchloric acid with chloric acid. Qualitative tests for chlorates were accordingly applied to the perchloric acid, and to the faulty precipitates obtained in the soil and fertiliser analyses, in every case with positive results.

Determinations of chloric and perchloric acids in the reputedly pure perchloric acid gave the following results (samples *A* and *B*) in comparison with that given by a satisfactory sample (*C*).

Table I. *Composition of samples of "perchloric" acid.*

Analytical reference No.	Sample of acid	$HClO_3$ %	$HClO_4$ %
483/13	*A*	20·50	0·40
431/150	*B*	6·93	16·79
431/149	*C*	0·29	23·89

The firm which supplied these samples subsequently explained that sample *A* had been supplied in error, and did in fact consist almost wholly of a solution of chloric acid instead of perchloric acid. The fact that sample *B* gave equally bad results shows, however, that the trouble was not merely due to the firm's error of labelling in the case of sample *A*, but is liable to occur with any sample of perchloric acid containing a moderate percentage of chloric acid, such as sample *B*.

The following results show the magnitude of the error which arises from the use of this impure perchloric acid[1].

The methods used for preparing the material for analysis were as follows:

Soils. The soil extract, prepared as recommended by the Agricultural Education Association(3), was treated as described by Davis(1, p. 56).

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[1] Many of the analyses here reported were carried out by Messrs G. C. Sawyer, T. Eden and A. H. Bowden, to whom acknowledgements are made.

Fertilisers. The official method for the analysis of salts of potash containing sulphates (4, p. 21).

Plant material. The material was ashed and the ash treated as described by Davis (1, p. 63).

Table II.

Estimations of potash in soils, fertilisers, and plant material.

		K₂O, calculated from the weight of precipitate obtained by using	
Analytical ref. no.	Material analysed	Good perchloric acid (Sample *A*)	Bad perchloric acid*
	Soils	%	%
480/10	Lydney Park: "Available"	0·0085, 0·0085	0·0926, 0·0969 (*A*)
480/14	Tongham, White Lane Farm: "Total"	0·29, 0·27	1·31 (*A*)
	Welwyn Garden City:		
480/38	"Total"	0·40, 0·42	1·93, 1·63, 1·39 (*B*)
480/39	"Available"	0·0076, 0·0068	0·0119, 0·0161, ·0212 (*B*)
	Fertilisers		
493/101–2	Kainit	14·23 (by platinum method)	32·50, 32·10, 30·64 (*A*); 24·60 (*B*)
431/171–2	Sylvinite	17·24, 17·44	(i) 29·39, (ii) 36·96 (*B*)
	Plant material		
480/36	Linseed	0·88, 0·85	2·03, 1·62, 1·28 (*B*)

* The sample of "perchloric acid" used is indicated by the letter (*A*) or (*B*) after the values in this column, reference being to the samples thus described in Table I.

The effect of successive washings with about 100 c.c. of 95 per cent. alcohol (saturated with potassium perchlorate) on the weight of the "KClO₄" precipitate is shown by the following figures, which were obtained by treatment of the precipitates from the analyses of sylvinite (Table II).

Table III.

Effect of successive washings on the faulty "KClO₄" precipitates.

	(i)	(ii)
	gm.	gm.
Original weight of "KClO₄" precipitate	0·8652	1·0868
Weight after first washing	0·7060	0·9274
„ second „	0·5562	0·7204
„ third „	0·4786	0·5782

These precipitates after their third washings were redissolved in water and treated with good perchloric acid (sample *C*) and concentrated in the usual way. After this treatment, their weights agreed with those originally obtained in the analysis of sylvinite using good perchloric acid (see Table II).

Table IV.

Effect of re-treatment of faulty " $KClO_4$ " precipitates,
with good perchloric acid.

	(i)	(ii)
	gm.	gm.
Weight of " $KClO_4$ " precipitates after third washing	0·4786	0·5782
Weight after re-treatment with good $HClO_4$	0·5022	0·5120
Original weight of $KClO_4$, precipitate from analysis using good $HClO_4$	0·5072	0·5131

The way in which the presence of chloric acid in the perchloric acid gives rise to trouble is tolerably clear. In the case of soil and plant analyses, the liquid to which the perchloric acid is added contains barium hydroxide, added to remove sulphates. This baryta gives barium chlorate with the chloric acid, and owing to the lower concentration of perchloric acid (as a result of which it is also difficult to induce fuming on concentration) a certain proportion of the barium chlorate escapes conversion to barium perchlorate. Barium chlorate is a powerful oxidising agent, and thus frequently gives rise to deflagrations on the addition of alcohol. It is moreover only sparingly soluble in alcohol and it therefore largely remains behind with the potassium perchlorate and is weighed as such. In the case of analyses of potash manure salts, the preliminary treatment removes all metals except potassium and sodium, which are present as chlorides in the liquid finally treated with perchloric acid. Here again, chloric acid appears to act as a diluent of the perchloric acid, preventing the complete conversion of sodium and potassium chlorides and chlorates into perchlorates; the precipitate obtained contains a plentiful amount of chlorates, as found by an examination of the alcoholic wash liquids. Potassium chlorate is only very slightly soluble in alcoholic perchloric or chloric acids, and sodium chlorate, although more soluble than the potassium salt, is not sufficiently so to remain completely in solution. The final precipitate therefore consists of a mixture of potassium perchlorate, potassium and sodium chlorates, and some unchanged chlorides; the whole of this is weighed as $KClO_4$.

When present in sufficiently high concentration, perchloric acid decomposes both chlorates and chlorides. Hence the re-treatment of the faulty precipitates with a good sample of perchloric acid results in a residue of pure potassium perchlorate of the correct weight.

It would appear very probable that the occasional failures of the perchlorate method are largely due to this cause, which has escaped notice owing to the non-recognition of chloric acid as an impurity in perchloric acid, and its omission from the usual tests for analytical reagents [2].

Using a satisfactory sample of perchloric acid, Davis showed quite

definitely that the perchlorate method was superior to the platinum chloride method; there is no reason to doubt Davis's conclusions, provided that, in view of the results described in the present paper, the perchloric acid used is free from chloric acid. This may be readily ascertained as follows:

One cubic centimetre of the acid, which should already have been submitted to the tests for chlorides, sulphates, etc. as laid down in the approved tests(2), is diluted with 20 c.c. of water, and a few crystals of ferrous sulphate are added. The liquid is boiled for a short while, acidified with nitric acid, and a few drops of silver nitrate solution added. No more than a faint turbidity should be produced (provided the original acid has been shown to be free from chlorides). The same method can be used for the quantitative determination of chloric acid in an impure sample.

The Addition of Calcium Carbonate to Soil Extracts in which Potassium is to be estimated.

Mention may also here be made of a small detail in the estimation of potassium in soils by the perchlorate method, in which improvement is possible. In the case of a soil originally deficient in calcium carbonate, it is customary, following Neubauer's instructions(5), to add 0·5 gm. of pure calcium carbonate to the soil extract before evaporating and igniting. since that author found that in the absence of sufficient calcium salts, the potassium in the ignited residue was liable to be only partially soluble in water. The use of such a relatively large amount of calcium carbonate results in the consumption of an unduly large amount of perchloric acid, since the whole of the calcium chloride present in the water extract must be converted to perchlorate before an excess of perchloric acid remains, as indicated by strong fuming on concentration. Pure perchloric acid, although, of course, much cheaper than platinum chloride, is nevertheless quite an expensive item when a large number of routine analyses is involved. There is, moreover, an increased danger of contamination of the $KClO_4$ precipitate should the washing with alcohol be at all limited.

There appears to be no necessity for so much calcium carbonate to be used; the possibility of using less without sacrificing accuracy, and of thereby economising in perchloric acid consumption, was investigated. The following are the results obtained with soils originally entirely deficient in carbonates, using in one case 0·5 gm. $CaCO_3$ and in the other 0·1 gm. $CaCO_3$.

Table V.

Analytical reference no.	Soil	Amount of $CaCO_3$ added gm.	"Total" K_2O found %	Amount of perchloric acid needed to induce fuming on concentration c.c.
525/6	Leadon Court: Boyce Field	0·5	0·50	7·5
		0·1	0·50	2·5
	New Meadow	0·5	0·54	7·5
		0·1	0·55	2·5

The question has not been tested on a variety of soils but the above results appear to indicate that considerable economy in perchloric acid may be effected by the use of 0·1 gm. of calcium carbonate instead of 0·5 c.c., without any sacrifice of accuracy.

SUMMARY.

(1) It is shown that the presence of chloric acid in the perchloric acid used for the estimation of potassium in soils, fertilisers, and plant material, by Davis's method(1) gives rise to very erratic and erroneous results. Every sample of perchloric acid should, therefore, be tested for freedom from chloric acid before being used for the estimation of potassium.

(2) Results are quoted which indicate that in the application of Neubauer's method of treatment of the soil extract to a soil deficient in carbonates, it is sufficient to add only 0·1 gm. of calcium carbonate to the extract instead of the 0·5 gm. generally used. A considerable economy of perchloric acid is thereby effected.

REFERENCES.

(1) DAVIS, W. A. The Estimation of Potassium, especially in fertilisers, soil extracts, and plant ashes. *Journ. Agric. Sci.* **5**, 52, 1912.
(2) List of Reagents for analytical purposes, with notes indicating the standards of purity regarded as necessary for analytical work, prepared by a special committee appointed by the Councils of the Institute of Chemistry and the Society of Public Analysts, etc. 1915.
(3) HALL, A. D. *Analyst*, November, 1900.
(4) Ministry of Agriculture, Leaflet No. 18. Revised, July, 1918.
(5) NEUBAUER, H. *Landw. Vers.-St.* **63**, 141, 1905.

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THE DETERMINATION OF POTASH IN SOILS.

By A. H. DODD.

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IN June, 1921, the writer undertook a series of soil analyses in connection with the field trials organised by Dr J. A. Hanley for the Department of Agriculture at the University of Leeds. The soils were of diverse kinds and included samples of most formations from the Carboniferous upwards, most of which were from poor unmanured grass land. They were of obviously low potash content.

It soon became apparent that the well-known perchlorate method for the determination of potash could not be used for all soils indiscriminately without modification. It is considered of interest to those soil chemists who have to carry out potash determinations only occasionally to give some account of the difficulties met with. They have generally neither the time nor the facilities to read the extensive literature which has appeared in various journals since 1831 or to experiment with the method.

This work was not commenced with any idea of publication. There was no reason at the outset to suspect the method as given by Davis[1] and described in the *Chemists' Year Book*[2]. The work described, therefore, does not follow a definite line of research.

An outline of the method originally used is as follows:

100 c.c. of the hydrochloric acid soil extract (equivalent to 5 gm. of air dry soil) or 500 c.c. of the citric acid soil extract (equivalent to 50 gm.) were evaporated down to dryness. 1 gm. of chalk was added during this process and if sulphate was shown to be present by a qualitative test 10 c.c. of saturated barium hydroxide solution was added also. The residue was ignited ("Neubauered") for 12 hours at slightly less than dull redness till all traces of carbonaceous matter were burnt off and complete insolubility of iron, etc. was insured. The sintered residue was leached six portions each 20 c.c. of hot distilled water, the washings being put through a small filter paper. This extract is referred to in the following remarks as "aqueous extract." It was invariably colourless and on standing a fine white scum of calcium carbonate appeared. A few cubic

[1] *Journ. Agric. Sci.* (1912), **5**, p. 52.

[2] *Chemists' Year Book* (1922). Section on Soil Analysis, ed. Sir E. J. Russell.

centimetres of dilute hydrochloric acid were added, followed by 10 c.c. of perchloric acid (sp. gr. 1·12). This quantity is much larger than that recommended by Davis; a much larger quantity than 2·5 c.c. had already been found necessary by Dr Hanley. After evaporating till fuming, 20 c.c. of 98 per cent. alcohol were added and the perchlorate filtered off on a Gooch crucible; the washing liquor was alcohol saturated with potassium perchlorate, about 50 c.c. being used.

Despite all precautions it was found that certain soils, on addition of alcohol to the perchloric acid concentrate, gave precipitates which were partially amorphous, or gave material with the perchlorate which was obviously not potassium perchlorate. At times the precipitate choked up the asbestos filter and it was found impossible to get the alcohol through. Neither in Davis's original paper nor in any recent work on soil analysis is any mention of such difficulties found. Dr Hanley was of the opinion that the mass could be rendered suitable for filtering by re-evaporation with fresh perchloric acid, care being taken that the hot sand bath did not touch the edge of the concentrate. Actually two concentrations were often carried out. Morris[1], however, recommends three. Further, it was Dr Hanley's invariable procedure to wash with alcohol till a further small wash did not appreciably diminish the weight. This procedure is quite impossible when the asbestos filter would not allow the alcohol through except after hours of pumping.

It may be mentioned in passing that there is no consensus of opinion as to the best washing agent. Davis originally used alcohol with ·2 per cent. perchloric acid and later suggested alcohol saturated with potassium perchlorate, which, according to Thin and Cumming[2] is perfectly satisfactory. Atkinson[3] proposes methyl alcohol containing 5 per cent. of perchloric acid (sp. gr. 1·12), in which it is stated that the sulphates and phosphates of sodium and magnesium are soluble.

Others, however, mindful of the fact that potassium perchlorate may be precipitated from the washing liquid if sodium perchlorate be present, have reverted to the use of alcohol with traces of perchloric acid, *e.g.* Baxter and Kobayashi[4] use absolute alcohol with ·1 per cent. $HClO_4$ (see also Keitt[5]). At all events Morris, following Gooch and Blake, finally used 100 c.c. of 98 per cent. alcohol to 1 c.c. of perchloric acid (sp. gr. 1·12), *i.e.* approximately 97 per cent. alcohol and ·2 per cent. $HClO_4$. It is a pity that this experimenter did not try the effect of

[1] Morris, *Analyst*, Oct. 1920, **45**, No. 535, p. 349. (A most important paper with bibliography.)   [2] *Journ. Chem. Soc.* 1915, **107**, p. 361.
[3] *Analyst*, 1921, p. 354.   [4] *Journ. Amer. Chem. Soc.* 1920, p. 735.
[5] *Journ. Ind. and Eng. Chem.* 1920, **12**, p. 276.

washing with methyl alcohol liquors as his work is probably the most thorough contribution to our knowledge of the process and is of the highest order of accuracy. If there are no disadvantages attached to the use of methyl alcohol wash liquors such as alcohol denatured with 5 per cent. methyl alcohol, as suggested by Baxter and Rupert[1], the fact that the sulphates of sodium and magnesium are soluble is of very great importance. No investigation was carried out on these points by the present author, but in certain of the analyses the acid wash liquor was used in place of the perchlorate wash liquor; no appreciably different results were obtained. There are widely differing opinions as to the proper amount of wash liquor to use. Davis did not consider it of much importance whether one used 30 or 150 c.c.; Thin and Cumming propose 30-60 c.c., whilst Morris and others restrict the amount as far as possible. Since the perchlorates likely to be met with are readily soluble in alcohol there seems no necessity to use a large amount especially as no amount of washing will remove sodium or calcium sulphates if present.

Since the author could not find any simple means of overcoming the chief difficulty, viz., the presence of insoluble bodies with the perchlorate, it became obvious that there was some serious defect in the preliminary treatment.

Careful blanks were carried out on the reagents. The specimens of perchloric acid were poor—one labelled A. R. contained ·75 per cent. of solid matter after ignition, another had perceptible quantities of chloric acid (?), for when alcohol was added to the perchloric acid concentrate the whole burst into flame.

The hydrochloric acid was changed as that used at first contained traces of sulphate. The purest alcohol obtainable was employed. The distilled water was always viewed with suspicion owing to the state of the Leeds atmosphere. When control experiments were carried out using 1 gm. of pure calcium carbonate and the usual quantities of the ordinary reagents a fine deposit was invariably found on addition of the alcohol. This amounted from a few tenths of a milligram to 1·5 mgm. This is, of course, a relatively enormous quantity (1·5 mgm. of precipitate corresponds to approximately ·01 per cent. of K_2O in an ordinary determination) but was unavoidable. This deposit is doubtless silica from the reacting vessels—quartz was not available—and sulphate from the water and from the gas fumes in the laboratory.

As a result of the preliminary work it seemed that sulphates must be the cause of the trouble. Sodium and calcium sulphates in the aqueous

[1] *Journ. Amer. Chem. Soc.* 1920, p. 2046.

extract would not be decomposed by perchloric acid; there seems to be no reference to this point in most accounts of the methods of determination of potash soils. [*It is generally assumed that barium hydroxide will remove the sulphate; a slight excess of barium not being thought deleterious.*] Morris notes the difficulty of keeping barium from the precipitate and Jarrell[1] also records the fact that barium "gets through." For this reason the author was very shy of using this reagent. Consequently barium hydroxide was only added to those soil extracts which gave a qualitative test for sulphate. Whenever barium hydroxide was added traces of barium were invariably found in the perchlorate precipitate. Further than that addition or non-addition of barium hydroxide had not the slightest effect on the nature of the precipitate. If the soil extract gave an impure perchlorate precipitate at the final stage, the addition of barium hydroxide to the extract before evaporation caused no improvement.

Certain of the perchlorate precipitates were analysed for SO_4'' by dissolving up in strong hydrochloric acid and precipitating the sulphate in the usual way with barium chloride. Generally the solution obtained in hydrochloric acid was slightly turbid—possibly due to presence of silica. When barium hydroxide had been added to the original soil extract it was always necessary to filter off the trace of barium sulphate found in the precipitate before determining the other sulphates—presumably those of sodium, calcium or magnesium. From six determinations, two of which were from "available" potash determinations, the amount of sulphate in the precipitate amounted to over 5 per cent. reckoned as $CaSO_4$. The magnitude of the error in "total" determinations is illustrated by Table I.

Table I.

Soil	Wt. of precipitated perchlorate and impurity	Wt. of $BaSO_4$ precipitated
Thornton le Beans. Boulder Clay	·0315	·0098
Skipton. Boulder Clay	·0132	·0077
Ainderby Steeple. Glacial Sand	·302	·0082

In order to summarise the results into one table the methods employed are given under six headings.

Method 1. This implies that the procedure was that already described as "official." Unless marked with an asterisk no barium hydroxide was added to the extract.

[1] *Journ. Amer. Assoc. of Agric. Chem.* 1915, **1**, 29; abs. *Journ. Soc. Chem. Ind.* 1915, p. 1170.

Method 2. The perchlorate precipitates obtained by addition of alcohol were filtered and washed. After drying they were dissolved in as little water as possible and the insoluble matter filtered out. A little water was used for washing but the final bulk did not amount to more than 25 c.c. The filtered extract was again concentrated with perchloric acid. By this means a large number of determinations were carried out on "total" potash which are not recorded here. If calcium sulphate is present the error remains and is too great to be corrected for in "available" determinations.

Method 3. In order to eliminate sulphate entirely barium chloride was used to precipitate sulphate from the original aqueous extract and the excess of barium chloride removed by two evaporations and concentrations with ammonium carbonate, the barium carbonate being removed by filtration after each. The ammonium salts were removed by concentration in a beaker to avoid "creeping" and the final concentration and ignition carried out in a silica basin. The residue was leached out with a little hot water, the extract filtered and the concentration with perchloric acid carried out in the usual way. This process is very tedious and there is a great risk of loss in removing ammonium salts.

Method 4. As the former is too long for routine work a short cut was tried by removing calcium as oxalate from the aqueous extract. The filtrate from the oxalate precipitation was concentrated with aqua regia, evaporated and ignited gently. It was hoped that the sulphate formed by double decomposition of the ammonium oxalate and calcium sulphate would be removed by evaporation without any back reaction in the solid phase. There seem some possibilities in this device but there was no time to carry out many determinations.

Method 5. It should be easily possible to remove sulphates from the actual perchlorate precipitate by the method described under "3." Several determinations were carried out but the process was abandoned as the ammonium perchlorate formed suddenly flares off in the final ignition to remove ammonium salts. There is probably a loss owing to the high temperature; and risk of losses through explosion[1].

Method 6. If the insoluble substance obtained along with the perchlorate were only calcium sulphate it should be possible to allow for this[2].

One has merely to precipitate the sulphate in the precipitate with

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[1] Cf. Joseph and Martin. *Journ. Chem. Soc. Ind.* 1920, p. 94 T.

[2] Morgan. *Journ. Ind. Eng. Chem.* 1921, p. 225.

acid and barium chloride in the usual way and from the weight of SO_4'' calculate the amount of presumed calcium sulphate in the original precipitate. In April, 1922, the author received a copy of the Norwegian Geological Survey pamphlet No. 108[1], in which E. Johnson discusses the methods available for the determination of potash in minerals. Referring to the perchlorate method he states[2]:

"Certain sulphates can influence the results—not Fe, Al, Mn and Mg sulphates, however, as these are soluble in alcohol. Ba and Sr sulphates are insoluble and remain back in the filter after washing out with warm water so that they can be eliminated from the result. Potassium sulphate is soluble in alcohol and will be converted into perchlorate with excess of perchloric acid. On the other hand sodium and calcium sulphates which are insoluble in alcohol but soluble in water are weighed as potassium perchlorate. When these are present the sulphate must be precipitated from the hot water extract (of the perchlorate precipitate) with barium chloride and hydrochloric acid and calculated to

$$2CaSO_4 . H_2O.$$
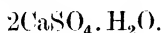
Because in, for example, cements...only a trivial error is caused by calculation of the sodium sulphate likely to occur, as $CaSO_4\tfrac{1}{2}H_2O$ — molecular weights 142 and 145 respectively."

In the table which follows the "allowance for $CaSO_4$" is based on a molecular weight of 136.

Cobaltinitrite Method. On the advice and information of Mr Lowson of the Department of Chemistry in the University of Leeds, this method was tried when the work was about half complete. A description of the method used is given at the end of this paper.

In the following table (Table II) all the results are drawn up together but the figures for any one soil were not necessarily obtained at the same time nor on the same extract.

Naturally only those soils which gave troublesome precipitates were experimented with. "Total" potash estimations which gave crystalline precipitates with only that amount of insoluble body as would be expected from the blanks were accepted.

Notes on Table II.

There are far too few experiments for definite conclusions except that agreement of duplicates by the "standard" method is no criterion that the results are correct. In the table there are only two comparisons

[1] *Norges Geologiske Undersökelse*, Nr. 108. "Glimmermineralernes Betydning som Kalikilde for Planterne." Goldschmidt and Johnson, Statens Raastofkomite Publikation, Nr. 8, Kristiania, 1922.

[2] *Om kvantitativ bestemmelse av Kali*, p. 67, by E. Johnson.

of the second method with others— in the first soil it is evident that the bulk of the impurity had been removed by this method and the figure obtained, ·012 per cent., is comparable with those in columns 6 and 7. That obtained by removing sulphate and excess of barium is evidently too low either owing to loss in manipulation or to adsorption of alkali ions by the three barium precipitates. It was realised that the second method is of interest to soil chemists as a short means of overcoming the sulphate difficulty when they are estimating potash in fertilisers or "total" potash in soils of known normal potash content.

Table II.

			Method						
			1	2	3	4	5	6	
No.	Soil — Parish and formation	Total or available	Standard method	Re-extracting and filtering precipitate	Removing sulphate and xs barium, etc.	Removing calcium as oxalate, etc.	Removing sulphate and xs barium from perchlorate	Allowing for calcium sulphate in ppt.	Cobaltinitrite method
1.	Bishop Burton (Wold): Cretaceous	Av.	·028	·012	·007	—	—	—	·009
			·037	—	—	—	—	·0095	
2.	Burton Agnes (Wold): Cretaceous	Av.	·023	—	·016	·015	—	—	—
			·032*	—	—	—	—	—	—
			·028*	—	—	—	—	—	—
			·025	—	—	—	—	—	...
3.	Pickering: Oolitic Limestone	T.	·61*	—	·076	—	—	·075	·052
			·58*	—	·08	—	—	—	·048
			·113	—	—	—	—	—	·050
			·17	—	—	—	—	—	
4.	,,	Av.	·019	—	—	·003	·007	·0085	·008
			·012	—	—	—	—	·008	
5.	Wykeham: Lacustrine (post-Glacial)	T.	·55	—	—	—	—	—	—
			·67	—	—	—	·31	—	·34
			·55*	—	—	—	—	·45	·36
			·63*	—	—	—	—	·47	—
			·57	—	—	—	—	—	
6.	Garforth: Coal Measures	T.	1·06*	·286	·146	—	—	—	·13
			·91	—	·124	—	—	—	
7.	Wykeham: Lacustrine (Alluvial)	Av.	·019	—	·014	—	—	·016	—
8.	Nostell: Coal Measures	,,	—	—	·015	—	—	—	·018
9.	Bramham: Magnesian Limestone	,,	·063	—	—	—	—	·052	·035
			—	—	—	—	—	—	·031
10.	Bolton Abbey	,,	·032	—	·0047	—	—	—	·0043

It is evident that the perchloric acid precipitate on re-solution in a small quantity of water can only be saturated with calcium sulphate which is only soluble to the extent of ·02 part per hundred, *i.e.* the sulphate taken up by a final bulk of 25 c.c. cannot be more than ·005 gm. This amount can be corrected for in the final calculation. This is only possible

in a "total" estimation, in which the perchlorate precipitate amounts to nearly ·1 gm.; in determinations of "available" potash the precipitate is so small that the error involved is too great.

Tests on a shallow soil from the Oolitic limestone (Nos. 3 and 4) were carried out most carefully for two reasons. The soil seemed abnormally low in potash for that district and the *extracts gave no qualitative test for sulphate.* The figure ·61 per cent. given by Method 1 for the "total" potash, was confirmed by a repetition; the cobaltinitrite determinations were carried out on a fresh extract prepared for the other determinations. In these, two and a half times the usual quantity of extract was employed and it will be noticed that the results are higher than the cobaltinitrite figure. When dissolved in water the perchlorate showed a faint turbidity.

The soil from Garforth was acid, having a lime requirement of 0·33 per cent. $CaCO_3$ and the sulphate present was due chiefly to Leeds' smoke. It will be observed that barium hydroxide had no effect in removing sulphate.

Generally speaking the removal of sulphate followed by removal of barium gave results comparable with those obtained by the cobaltinitrite method.

DISCUSSION OF RESULTS.

In view of the above results the following conclusions are drawn.

The presence of sulphates in a soil may vitiate the results obtained by the standard procedure. It is not sufficient to test the soil qualitatively for sulphate in order to decide whether or not it is necessary to add barium hydroxide at the initial stage. The qualitative test for sulphates by adding barium chloride to the acidified soil extract is not delicate enough; in presence of a large excess of other salts and organic matter the sulphate may not be precipitated. Further, *organic* sulphur is taken up in the soil extract and is oxidised to sulphate in the subsequent treatment of the extract.

If sulphates are present, addition of barium hydroxide to the hydrochloric or citric acid extracts is useless to remove them. Firstly the sulphate precipitated as barium sulphate on adding barium hydroxide to the extract is converted back into calcium sulphate when the evaporated extract is ignited according to the Neubauer method; carbonates are formed during the ignition and the conversion of calcium carbonate into calcium sulphate is to be expected. The following reaction in the dry phase can easily be demonstrated:

$$CaCO_3 + BaSO_4 \rightleftarrows BaCO_3 + CaSO_4.$$
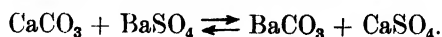
Moreover, the carbonaceous matter in the ignited mass may be instrumental in the formation of sulphates in the subsequent treatment, as follows. As is well known sulphates are reduced by ignition with carbon when barium sulphate is ignited in contact with filter paper; in ordinary analytical work due allowance has to be made for the reduction of sulphate to sulphide. Hence sulphates in the residue which is obtained on evaporation the soil extract may be reduced to sulphides by the carbon formed in breaking up of the organic matter during the subsequent ignition. These sulphides are leached out with water and appear in the aqueous extract. Immediately perchloric acid is added they are oxidised to sulphates. There are also other considerations. Hirst and Greaves[1] have discussed in great detail the solubility of barium sulphate in alkali salts. They point out that it is not so easy to precipitate barium sulphate completely as is generally supposed. It does not seem very likely that the barium sulphate left behind in the ignited soil extract would be appreciably soluble in the water used for leaching out the soluble salts. However, the possibility remains and cannot be disregarded when one is dealing with small amounts of precipitate.

Morris emphasises the possibility of excess of barium in the solution being converted into sulphate by the gas fumes in the laboratory. It is obvious that under ordinary conditions this is difficult to avoid. The cumulative effect of these possible sources of sulphate must be considerable. It may be relevant to mention in this connection that Dr Hanley avoids adding barium hydroxide to the raw soil extracts to remove sulphate so that any complications introduced by ignition of barium sulphate in the dry phase are ruled out. He removes sulphate from the aqueous extract. Experience, however, has shown that barium sulphate nevertheless appears in the perchlorate precipitate. This may be attributed to (a) solubility of barium sulphate in alkali salts, (b) the formation of colloidal barium sulphate, (c) formation of sulphate by the excess of barium taking up sulphuric acid from the vitiated air of the laboratory. In this process there may be a loss of potassium by adsorption or occlusion in the barium sulphate precipitate and in the foregoing experiments in which excess barium was removed from the aqueous extract after removing sulphate an undoubted error is caused by this adsorption. This, however, is not observable by any defect in the precipitate[2].

[1] *Soil Science*, **13**, No. 4, p. 221.

[2] Methods have been studied in order to improve the precipitation of barium sulphate such as precipitation under pressure or in "extreme dilution." Cf. Hahn and Otto, *Journ. Soc. Chem. Ind.* 1923, pp. 427 A, 962 A.

Hence the soil chemist is faced by all these actual difficulties and possible sources of error. It is not always practicable in an ordinary agricultural laboratory to use quartz vessels, electric evaporators and to adopt the technique necessary to get results free from suspicion.

The author is therefore strongly of the opinion that the cobaltinitrite method should replace the perchlorate method as the official method for soil analysis. It has been claimed that by this method it is possible to estimate accurately the potash in 1 c.c. of blood. For small quantities of potash the Norwegian pamphlet recommends this process.

For that reason a brief résumé of the method finally adopted is given.

Cobaltinitrite Method for Potash Determination.

The process used is substantially that of Mitscherlich as modified by Christensen and Feilberg[1], in whose paper a résumé of the most important work on the subject has appeared. The precipitation is carried out in the way described by Mitscherlich except that excess of sodium chloride is used in solution. It was desirable to shorten the three preliminary ignitions. Mitscherlich recommends concentration of the soil extract with dilute acids and ignition, followed by treatment with a few drops of pure sodium carbonate and re-ignition. The residue is then neutralised with dilute nitric acid and again ignited. The preliminary experiments showed that the following simplified procedure worked satisfactorily.

The aliquot portion of soil extract was concentrated down in the usual way (nitric acid was added to the acid extracts) and the mass ignited over a full Bunsen flame for six hours. The sintered mass was leached out with about 100 c.c. of water and the filtered extract evaporated in a glass dish with a few drops of dilute hydrochloric acid. This residue was extracted with water, filtered, and concentrated to 10 c.c. 10 c.c. of 10 per cent. cobalt chloride solution, 5 c.c. of 10 per cent. sodium nitrite solution and 5 c.c. of saturated common salt were then added. The solution was evaporated down to dryness on a water bath, the mass being constantly stirred with a glass rod to avoid formation of crusts. A little glass dust facilitates this, as recommended by the above authors. The mass is then worked into a fine state of division with 3 c.c. of 10 per cent. acetic acid, a few cubic centimetres of water are added and the whole is filtered through a Gooch crucible. The solution often filters badly and instead of an asbestos filter, filter paper covered with a layer

[1] Christensen and Feilberg, *Landw. Stat.* **97**, p. 27.

of glass dust has been suggested (Christensen and Feilberg); two thicknesses of good filter paper intended for barium sulphate precipitates will, however, hold the cobaltinitrite quite well. The lower circle should be slightly larger than the upper so that it can be well pressed round the side under pressure, being washed with the 2·5 per cent. sodium sulphate solution (the wash liquor used) at the same time. The subsequent treatment followed the usual lines except that for titration N/20 permanganate was used, 1 c.c. of which corresponds to ·0047 gm. K_2O.

The following are typical results.

Duplicate portions of three different soils were taken and to one (b) of each ·00254 gm. of K_2O were added, in the form of KCl. The values obtained for the percentage of K_2O in the soil were as follows:

"Total"	Parlington (Coal Measures)	Parlington (Magnesium Limestone)	Kipling Coates (Wold Soil, Chalk)
(a) % K_2O	·342	·231	·193
(b) % K_2O	·340	·230	·196
(After deducting that added as KCl)			

In another series potash was actually determined in the perchloric acid precipitates obtained by the standard procedure. Soil: Pickering (Oolitic Limestone). "Total" potash.

(a) Cobaltinitrite method, as above   ...   .   ·052 %

(b) Same method but ·0127 gm. K_2O added to extract   ·048 %
    This deducted before calculating result.

(c) Same method on actual perchlorate precipitate after
    evaporating to dryness and ignition with excess hydro-
    chloric acid   ...   .   ·050 %

The values found by the perchlorate method are given in Table II. The above agreement is quite satisfactory. Soil: Bramham Park, Magnesian Limestone. "Total" potash.

(a) Cobaltinitrite method, as above   ...   .   ·096 %

(b) Same method on actual perchlorate precipitate   ...   ·098 %

A perchlorate estimation was carried out on the same extract sulphate being removed from the aqueous extract and excess of barium removed by two precipitations with ammonium carbonate. The value found by this process, which took about a hundred times the length of time needed for the cobaltinitrite method was ·104 per cent.

Consistent results were always obtained.

The disadvantages of the perchlorate method are

(*a*) it is costly—costlier than even the platinum method;

(*b*) requires considerable knowledge of the difficulties and knowledge of the literature before consistent results can be obtained;

(*c*) even in the hands of the expert errors can creep in owing to the impossibility of knowing the composition of the soil extract. To escape these the process is extraordinarily long.

The cobaltinitrite method has the advantages of being

(*a*) cheap;

(*b*) very simple to carry out, the process being volumetric;

(*c*) unaffected by small quantities of other substances; the presence of sodium is no disadvantage. Apart from the evaporation the process is very quick.

The cobaltinitrite method can no longer be considered in the experimental stages and it will be to the great advantage of soil chemists when it is regarded as "official."

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ON SOME PHYSICAL PROPERTIES
OF TRANSVAAL SOILS.

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(With Five Text-figures.)

INTRODUCTION.

MUCH work has been done in the past on the chemical composition of South African soils. The study of the physical properties of soils has, however, been somewhat neglected.

Transvaal soils differ from those of European countries in several important respects. In the first place, a large proportion of the soil is derived *in situ* from igneous rocks, and secondly, the country has never been glaciated in recent geological times. These two factors bear directly on the physical properties of the soils. Soil formation takes place chiefly by the chemical decomposition of rocks and only to a limited extent by mechanical disintegration. As a consequence we find in our soils very fine material, resulting from the chemical decomposition of easily weathered minerals, and rather coarse material which represents those rock constituents which are only subject in a limited degree to decomposition. In comparison with European soils particles of intermediate size do not play any dominant part in the mechanical make-up of Transvaal soils. Soils of alluvial origin are, of course, excepted. These latter occupy only a small area, but they are of considerable importance in the present stage of development of the country. Another factor which differentiates Transvaal soils from European ones is the presence in many of the former of finely divided hydrated ferric oxide. This feature will be discussed later.

In this paper some data on the physical properties of Transvaal soils are presented and their relation to the mechanical composition, texture, and origin of the soils is discussed.

I. THE RELATION BETWEEN THE CLAY CONTENT AND HYGROSCOPIC
WATER AND LOSS ON IGNITION.

In Transvaal soils the percentage of organic matter is rather low
as a rule and loss on ignition represents to a large extent water of hydration
and other volatile matter. A study of the results of the analysis of 88
samples, representing almost every soil type occurring in the Transvaal
showed that there is a fairly steady relationship between the percentage
of clay and the combined percentages of hygroscopic moisture and loss
on ignition. This relationship is shown graphically in Fig. 1. The points

Clay per cent.

× —— Moisture plus loss on ignition   o - - - Loss on ignition   + · · · · Moisture
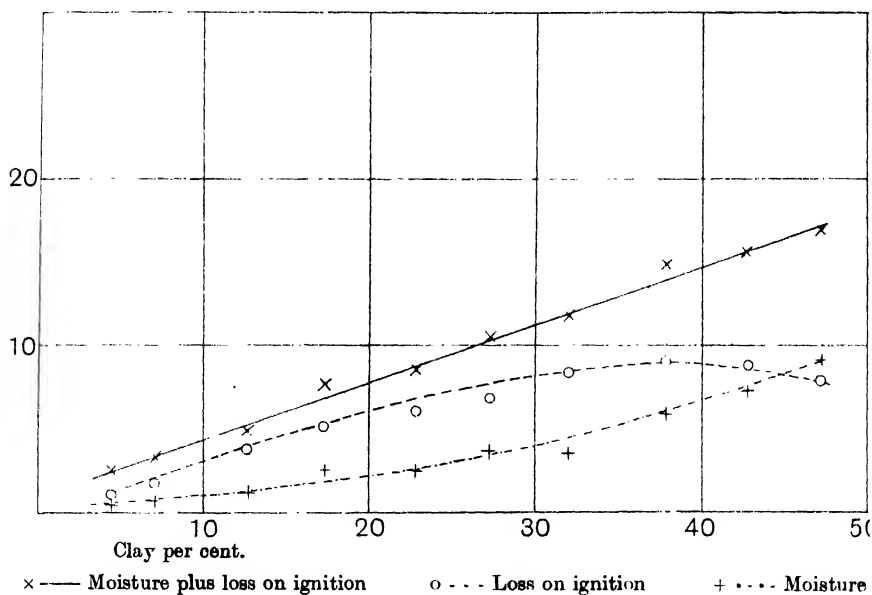
Fig. 1.

plotted were obtained by averaging (*a*) the percentage of clay falling
within the groups 0–5 per cent., 5–10 per cent. etc., and (*b*) hygroscopic
water and loss on ignition for soils having more or less the same clay
content. It would seem that this variation of hygroscopic water plus
loss on ignition with clay content is best represented by a straight line
and that a factor can be derived by means of which the approximate
percentage of clay can be calculated if the percentages of hygroscopic
moisture and ignition loss are known. This factor is approximately 2·5.
Thus a soil retaining, say, 4 per cent. of hygroscopic water and having
an ignition loss of 6 per cent. may be assumed to contain about 25 per
cent. of clay.

It is scarcely necessary to remark that there are exceptions to this generalisation. These exceptions fall into two classes. In the first we have soils rich in organic matter above the average, and in the second we have highly ferruginous soils which show on mechanical analysis much higher percentages of clay than one would suppose them to contain judging from their behaviour in the field. This peculiarity of ferruginous soils has been discussed on previous occasions (4, 10). We can, however, easily allow for these exceptions though not on a strictly quantitative basis. If we find by a preliminary hand and eye examination that the soil contains an amount of organic matter above the average, we may safely conclude that the clay content is less than $2 \cdot 5 \times$ hygroscopic moisture + loss on ignition. If, on the other hand, we are dealing with a highly ferruginous soil we may assume that the content of clay *as shown by mechanical analysis* is greater than that calculated as above. It is quite evident that the finely divided hydrated ferric oxides which form such a considerable portion of the "clay" fraction do not behave as clay.

On plotting curves for hygroscopic water and loss on ignition separately it is found that there is decided increase in the ratio $\dfrac{\text{moisture}}{\text{clay}}$ and a decided decrease in the $\dfrac{\text{loss}}{\text{clay}}$ ratio, the bending of the latter curve towards being rather sharper than the bending of the former away from the clay ordinate. The proportionate decrease of loss on ignition with increase in clay content is probably due to the fact that the loss on ignition is made up of at least two factors, the organic matter and the water of hydration. If we assume that the latter increases directly with the clay we must conclude that the organic matter cannot increase with clay content beyond a certain limit. This conclusion is probably a correct one. The two curves are shown in Fig. 1 where the actual points plotted are given. In view of the variations in the actual figures, omitted here for reasons of space, the curves must be regarded as showing the general trend rather than representing any definite relationship.

II. EXPERIMENTAL METHOD.

The method adopted for the determination of water capacity and of pore space was that of Keen and Raczkowski (3) modified in certain details. The modified procedure was as follows.

The apparatus consists of cylindrical brass sieves, the capacity of which has been accurately determined by plugging the holes, filling with water, sliding a glass plate on top, taking care to exclude all air bubbles,

and weighing. The sieves, together with a disc of filter paper which covers the holes in the bottom, are weighed, filled with soil in as uniform a manner as possible, and weighed again. They are then placed in water to a depth of about 20 mm., the sieves being 37 mm. deep, and allowed to stand overnight. The sieves are then removed from the water, dried on the outside, and weighed. The excess of soil, *i.e.* that which has been expanded above the edge of the sieve, is removed by means of a sharp spatula, transferred to a weighed dish, and weighed. The sieve with the residual wet soil is also weighed. Both portions are then dried in the steam oven to constant weight.

The chief modifications found necessary were the following. In certain soils the expansion is so great that dry soil is pushed half a centimetre or more above the edge of the sieve so that some of it falls over the edge and is lost. To prevent this loss a piece of thin copper foil is attached to the sieve in the form of a cylinder, held in place by a rubber band and projecting well above the edge of the sieve. The foil is weighed together with the excess soil scraped off and also dried therewith and weighed again. The weight of the foil is known. The drying of the residual soil in the sieve sometimes takes longer than the 24 hours prescribed by Keen: for this reason this drying is continued until weight is constant.

A departure from the procedure of Keen and Raczkowski is made in the preparation of the sample. In the work cited soils were used of which the whole passed a 100-mesh sieve. Such a procedure is, of course, out of the question in the case of soils of coarse texture. The suggestion was made that such soils should be crushed with the wooden pestle and passed through the 100-mesh sieve and that the portion failing to pass the sieve be then re-mixed with that passing through. This procedure was adopted but a millimetre sieve was substituted for the 100-mesh, as the labour entailed in passing certain of our soils through the smaller sieve (without grinding coarser particles) is enormous and furthermore the finely pulverised soil would be less representative of the field soil than that crushed to pass a millimetre sieve.

In the case of the coarse sandy soils the agreement between duplicates may be regarded as satisfactory except in the determination of "expansion per unit volume" in which the agreement is poor. The differences between duplicate determinations of water capacity and pore space were on the average 1·8 per cent. and 1·1 per cent. respectively of the determined quantities, the maximum differences being 4·7 per cent. and 3·3 per cent. respectively.

A few of the worst results are given below:

Table I.

Sample No.	1901	2211	2729	3985	4086	5263
Water capacity	(22·7	46·3	29·7	31·8	28·4	29·9
	(21·9	44·8	28·7	30·7	27·5	31·3
Difference	0·8	1·5	1·0	1·1	0·9	1·4
	= 3·6 %	= 3·2 %	= 3·4 %	= 3·6 %	= 3·0 %	= 4·7 %
Pore space	(34·4	48·9	41·3	41·3	39·3	39·8
	(33·4	48·0	39·9	40·3	38·2	40·6
Difference	1·0	0·9	1·4	1·0	1·1	0·8
	= 3·0 %	= 1·8 %	= 3·3 %	= 2·5 %	= 2·7 %	= 2·0 %

It must be clearly understood that the results above quoted are not representative and that they are deliberately selected as the worst six results obtained by us. In about a third of the determinations of water capacity and in about two-thirds of the determinations of pore space duplicates differed by 1 per cent. or less.

III. The Relation between the Clay Content and the Water Capacity and Pore Space.

The results obtained for 48 soil samples, representing most of the important soil types occurring in the Transvaal, are plotted in Fig. 2, the results being averaged, as before, for soils containing less than 5 per cent. of clay, 5 per cent. to 10 per cent., and so on. The increase of pore space with increasing clay content would appear to be best represented by a straight line, while the increase of water capacity with increasing clay content is perhaps best represented by a curve.

If p grams of soil absorb q grams of water

$$\text{Water capacity} = \frac{q}{p} \times 100,$$

$$\text{and pore space} = \frac{q}{q + \dfrac{p}{\text{sp. gr.}}} \times 100.$$

Pore space is greater than, equal to, or less than water capacity according as p is greater than, equal to, or less than $q + \dfrac{p}{\text{sp. gr.}}$.

If the relation between pore space and clay be represented by a straight line then that between water capacity and clay must be represented by a curve, provided specific gravity is constant. In the figure water capacity is calculated on the basis of the soil originally taken while pore space is calculated on the soil left after the portion which

expands beyond the dimensions of the sieve has been removed. If both values are calculated on the latter basis the relation is not materially affected though somewhat lower values are usually obtained for water capacity, as has been noted by Keen and Raczkowski.

Clay per cent.

o——— Pore space   + - - - Water capacity
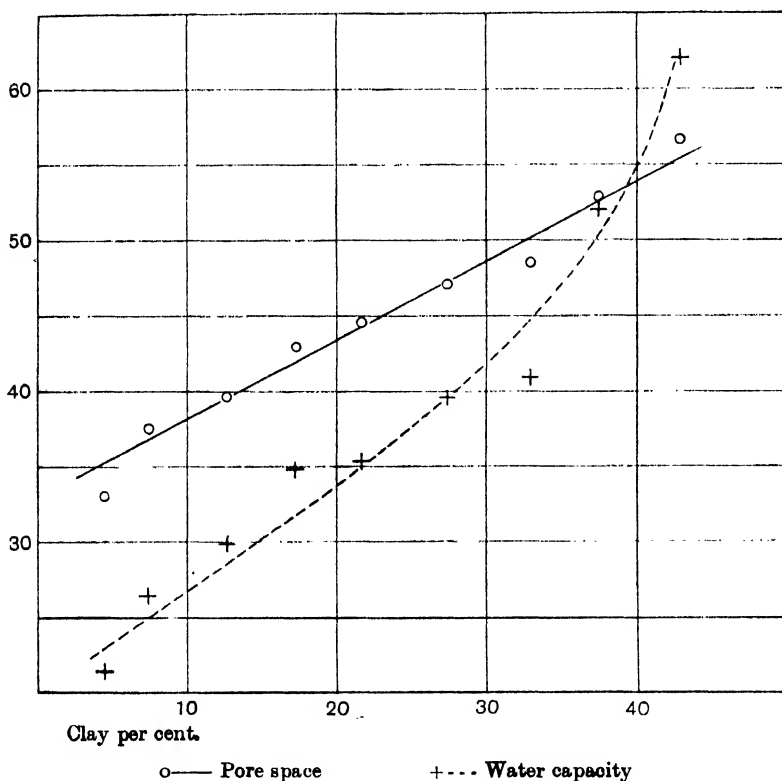
Fig. 2.

In most works on soil physics pore space is defined as the space between the soil particles which is occupied by air and by water. This in the dry soil is given by the expression

$$\frac{\text{true specific gravity} - \text{apparent specific gravity}}{\text{true specific gravity}} \times 100.$$

The definition of pore space must be modified according to the method adopted for its determination. In Keen and Raczkowski's procedure pore space is the space between the soil particles which is occupied by water *when the soil is saturated with water.* This qualification is important.

Pore space as determined by this method does not agree with that calculated from the above formula, being higher in all cases when the "true specific gravity" determined by the sieve method is used in the calculation, but in some cases higher and in others lower when the true specific gravity as determined by the bottle method is used. The difference between the determined pore space and that calculated from true and apparent specific gravities found by the sieve method is due to the method of calculation as the following considerations show.

If we consider the vessel filled with saturated soil and take V as the volume of the vessel and v as the actual volume of the soil, we have

$$\text{Pore space} = \frac{V - v}{V} \times 100 \qquad \ldots\ldots(1).$$

On the other hand if W is the weight of soil originally taken and w the weight left in the vessel after the excess soil has been removed, we have

$$\text{Apparent sp. gr.} = \frac{W}{V},$$

and

$$\text{True sp. gr.} = \frac{w}{v};$$

so that using these values to calculate pore space we have

$$\text{Pore space} = \frac{\dfrac{w}{v} - \dfrac{W}{V}}{\dfrac{w}{v}} \times 100 = \left\{ \frac{V - v}{V} - \frac{v(W - w)}{Vw} \right\} \times 100 \ldots(2).$$

Expressions (1) and (2) differ only by the quantity $\dfrac{v(W - w)}{Vw}$ which can be evaluated on any particular case. $W - w$ is the weight of the excess soil scraped off plus that of the water originally contained in the soil taken; the other symbols have already been defined. A few examples are given in the last two columns of Table II. The agreement is not exact because the quantity $W - w$ is determined by weighing the excess of soil and not merely subtracting w from W.

The values for pore space calculated from the sp. gr. determined by the usual bottle method, are considerably higher than those calculated from the so-called "true specific gravity" determined according to the method of Keen and Raczkowski. Table II also shows that for soils which do not expand greatly on wetting the figures in column (a) are also greater than those in column (c), but in the case of soils which expand considerably the latter method gives much higher results than

the former. Thus in samples Nos. 5706, 2729, 3985, 5347, 2039, 4881 and 5600 the determined pore space is less than that calculated from the true specific gravity by the bottle method, for samples Nos. 5531, 4948, 3614 and 5344 the former is greater than the latter, while for one sample, No. 2211, these values are practically identical. The differences cannot, therefore, be completely accounted for by supposing that the air is not entirely displaced by water. In those soils in which the volume expansion is great it is obvious that the soil particles do not occupy the same actual volume when wet as they do when dry. Not only does the pore space become filled with water but some of the soil particles apparently absorb water and swell like gelatine when soaked in water. Probably there is an increase in the degree of dispersion of the soil colloids.

Table II.

			Pore space				
Sample no.	Clay	Volume expansion	Calculated from true sp. gr. by bottle method (a)	Calculated from "true" sp. gr. by sieve method (b)	Determined (c)	Difference between (b) and (c)	$\dfrac{v(W-w)}{Vw}$
	%	%	%	%	%	%	%
5706	4·7	0·4	42·9	35·3	36·1	0·8	0·5
2729	8·3	0·9	46·8	39·6	40·6	1·0	0·9
3985	11·5	2·3	48·1	39·0	40·8	1·8	1·8
5347	17·5	6·2	50·0	39·9	43·9	4·0	4·2
2211	23·2	17·8	48·8	38·1	48·5	10·4	10·2
2039	31·0	6·3	51·9	41·3	46·0	4·7	5·5
3537	34·3	8·1	54·0	43·8	48·7	4·9	5·8
5531	36·6	30·0	49·8	40·1	56·5	16·4	16·5
4881	38·3	7·0	52·1	44·5	48·3	3·8	4·9
4948	39·4	43·7	51·3	38·3	59·8	21·5	20·8
3614	41·7	38·7	49·8	37·8	56·8	19·0	19·6
5344	42·3	45·3	49·3	39·2	60·8	21·6	21·3
5600	44·8	6·3	56·8	46·9	52·4	5·5	5·1

The values for pore space calculated from the apparent and true specific gravity bear no apparent relation to the texture; this is illustrated graphically in Fig. 3, which is plotted in the same manner as Fig. 2.

If we represent the relation between the clay content of a soil and its pore space[1] by a straight line it must be possible to calculate either of these quantities if one is known. The line drawn in Fig. 2 to represent this relation is

$$x = 1 \cdot 9y - K,$$

[1] Here, and subsequently in this paper where not otherwise stated, the term pore space is used in the sense defined by the method of determination proposed by Keen and Raczkowski.

where $K = 62.4$. For the nine different groups of soils the value of K varies from 58·2 to 64·8 and for individual soils from 46·7 to 74·2, so that it would appear to be useless to attempt to calculate, say, clay from pore space. It was found, however, that the constant K did not vary so much for soils of similar character and origin as it did for the whole number of samples. The heavier soils comprised so-called "black turf," red ferruginous soils, and grey soils. For the black turf the most suitable value for K was found to be 71, for the red ferruginous soils, 58·1. Only two grey heavy soils were examined so that no general value for K could be taken. If we use the above values to calculate clay from pore space we get the results given in Table III.

o - - - Calculated from true sp. gr. by bottle method.
x - - - Calculated from "true sp. gr." by Keen's method.
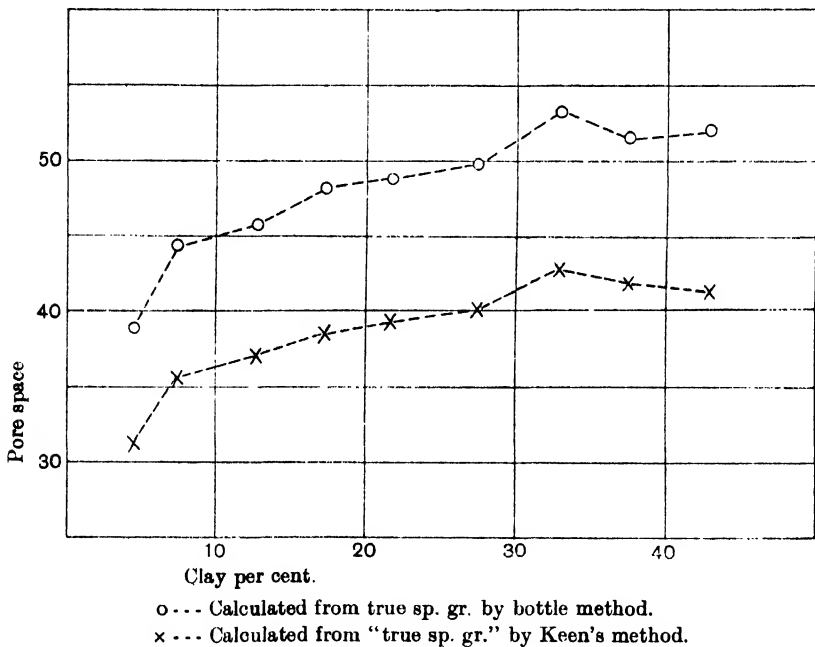
Fig. 3.

The agreement between the calculated and the determined values is quite fair. It was not found possible to apply this method with success to any other groups of soils. Sandy soils derived from the Bushveld granite and Pretoria quartzite require a value lower than the mean, while for sandy loams and loams the mean value is perhaps the most suitable. As is the case with the relation of moisture plus loss on ignition to clay, soils containing much organic matter give considerably higher values for pore space than do soils of similar clay content having only

an average proportion of organic matter. Calculation of the pore space from the clay content gives much more satisfactory results than the converse operation but here, too, the nature of the soil affects the results.

Table III.

Red ferruginous soils Clay = 1·9; pore space − 58			Black turf soils Clay = 1·9; pore space − 71		
Sample no.	Clay		Sample no.	Clay	
	Calculated	Found		Calculated	Found
3684	21·4	20·9	5287	28·0	26·5
4878	21·4	21·7	5531	36·3	36·6
3477	27·9	25·3	4948	42·6	39·4
3658	25·6	29·0	3614	36·8	41·7
1830	31·1	29·1	5344	44·5	42·3
2039	29·4	31·0	—	—	—
5349	35·3	32·4	—	—	—
3670	36·4	34·1	—	—	—
3537	34·5	34·3	—	—	—
2034	40·6	36·0	—	—	—
3632	35·3	38·1	—	—	—
4881	33·8	38·3	—	—	—
5600	41·6	44·8	—	—	—

If we take the equation

$$x = 1·9y - 62·4$$

as the relation between clay and pore space it follows that the relation between water capacity and clay is given by the expression

$$\text{Water-capacity} = \frac{x + 62·4}{(127·6 - x)\,S},$$

where S is the specific gravity of the soil. In Fig. 4 water capacity determined on the residual soil, and calculated as above, are plotted against clay.

Pore space may be calculated from moisture and loss on ignition by combining the equations relating these two values to clay. The results for the groups averaged as before are given below:

Table IV.

	Clay	Pore space Determined	Pore space Calculated
Groups	%	%	%
I	4·5	33·0	35·5
II	7·4	37·5	37·2
III	12·7	39·6	39·1
IV	17·3	42·9	42·1
V	21·7	44·5	43·6
VI	27·4	47·0	45·5
VII	32·9	48·4	50·5
VIII	37·5	52·8	51·3
IX	42·9	56·7	55·5

These and similar calculations are interesting but are not of much practical value as they break down when applied to individual soils in at least 50 per cent. of cases.

The percentage of clay in a soil has been taken as the measure of its structure but equally good relationship can be worked out for fine gravel and sand. This follows from what has been said above regarding the subordinate part played by the silt fractions, since the percentages of fine gravel and sand taken together vary inversely as the percentage of clay.
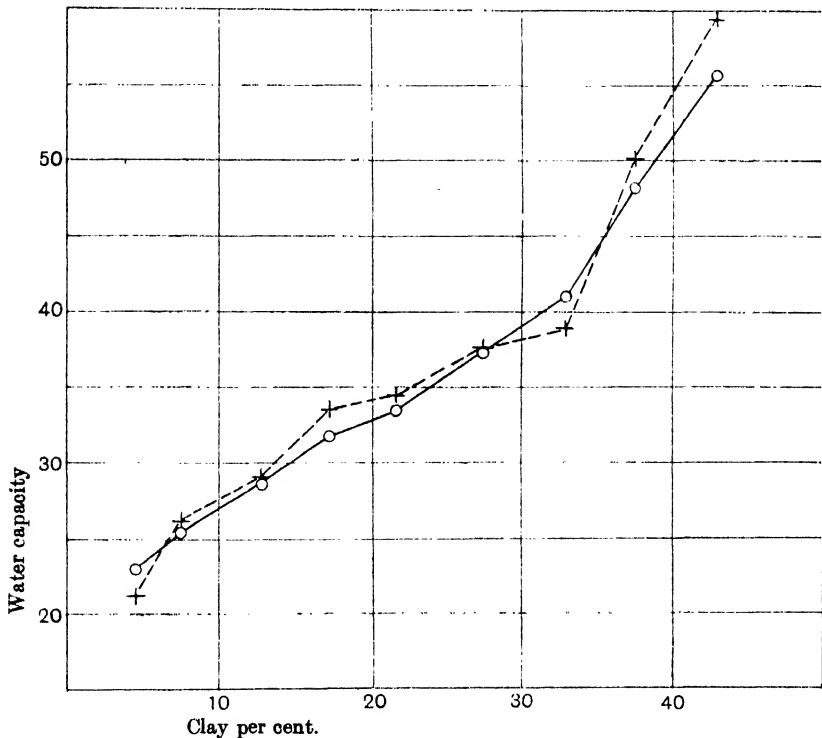
+ - - - Determined on residual soil.   o —— Calculated from clay content.

Fig. 4.

In the majority of Transvaal soils the percentage of silt fractions follows that of the clay fraction so closely that the use of, say, the total percentage of particles below 0·01 mm. in diameter, *i.e.* the silt fractions plus the clay, as a basis of comparison instead of the clay fraction only does not materially alter matters.

It may be assumed, in the majority of cases where soils of similar clay content are compared, that that soil which contains the most silt

will have the higher pore space, water capacity, and volume expansion, but this is by no means general as may be seen in the following table in which the mechanical analyses of three pairs of soils are given together with their pore space, water capacity, and volume expansion. One of each pair contains much more silt than the other while the clay content is more or less the same.

Table V.

Sample no.	1982	5287	5349	2039	2211	3477
	%	%	%	%	%	%
Moisture	3·53	5·56	4·45	4·04	3·18	2·67
Loss on ignition ...	6·93	5·68	9·22	9·33	7·76	4·93
Fine gravel ...	1·6	2·5	2·3	10·8	0·7	6·5
Sand	17·3	26·2	9·1	14·0	20·6	36·5
Fine sand ...	15·5	21·8	19·2	15·9	19·2	12·8
Silt	10·7	5·2	8·3	6·2	11·5	6·0
Fine silt	11·1	3·4	10·8	3·8	5·4	2·2
Very fine silt ...	5·5	3·2	5·7	4·8	5·6	3·1
Clay	27·0	26·5	32·4	31·0	23·2	25·3
Pore space ...	47·0	52·1	49·1	46·0	48·5	45·2
Water capacity ...	40·1	53·5	42·7	38·5	45·6	31·1
Volume expansion	9·9	26·1	3·5	6·3	17·8	7·2

In two cases out of three the soil with the higher silt content shows the higher pore space and water capacity, but in the third case the converse is found; consequently it would be impossible to introduce a factor dependent on silt content in calculating water capacity or pore space from the percentage of clay.

IV. Comparison of the Properties of Heavy Red, Grey and Black Soils.

A relation between an important property such as pore space and mechanical composition would do much towards placing the interpretation of mechanical analysis on a sound basis. The rough relationship discussed above only serves to emphasise the necessity for extreme caution in making deductions from the results of mechanical analysis.

The heavy soils, that is, soils which contain much clay as found by mechanical analysis, occurring in the Transvaal may, as previously stated, be roughly divided into three groups according to colour, viz. red, grey and black. The grey and black soils swell up enormously when wetted, thus showing a high volume expansion, while the red soils do not expand to any great extent. The latter have a much lower water capacity and pore space than the former. The field behaviour is also quite different. The red soils assume under proper cultivation a granular structure and some of them have even been described by casual observers as sandy soils.

In Table VI the physical properties of some red, grey, and black soils of similar clay content are compared. Attention is drawn to the striking differences, particularly in water capacity and volume expansion, between the red soils on the one hand and the grey and black soils on the other. Reference to the mechanical analyses of these soils, given in Table VII, shows that these differences may, in the case of the grey soils, possibly be accounted for by the proportion of silt which they contain.

Table VI.

Sample no.	Clay %	Moisture and loss on ignition %	Pore space %	Water capacity %	Volume expansion %	Colour
4878	21·7	6·7	41·8	26·4	4·1	Red
2211	23·2	10·9	48·5	45·6	17·8	Grey
3658	29·0	6·7	44·0	32·7	6·2	Red
1982	27·0	10·5	47·0	40·1	9·9	Grey
5287	26·5	11·2	52·1	53·5	26·1	Black
3632	38·1	11·4	49·1	39·3	8·6	Red
1865	36·5	11·7	51·5	49·4	24·1	Grey
5531	36·6	14·9	56·5	60·6	30·0	Black
4948	39·4	17·5	59·8	75·2	43·7	Black
5600	44·8	15·1	52·4	48·3	6·3	Red
5344	42·3	16·8	60·8	72·3	45·3	Black

Table VII. *Mechanical analyses.*

Sample no.	4878 %	2211 %	3658 %	1982 %	5287 %	3632 %	1865 %	5531 %	4948 %	5600 %	5344 %
Moisture	1·55	3·18	2·03	3·53	5·56	2·31	4·69	7·68	7·57	4·32	7·91
Loss on ignition	5·15	7·76	4·70	6·93	5·68	9·10	6·96	7·26	9·93	10·75	8·94
Fine gravel	6·1	0·7	4·0	1·6	2·5	2·9	0·2	1·2	0·3	2·5	0·2
Sand	39·5	20·6	33·6	17·3	26·2	17·0	15·2	19·8	6·5	5·1	7·7
Fine sand	16·0	19·2	15·9	15·5	21·8	11·4	19·4	15·0	15·1	9·6	17·4
Silt	4·9	11·5	3·8	10·7	3·4	5·9	4·7	6·3	8·8	8·6	8·8
Fine silt	3·8	5·4	4·1	11·1	3·4	9·4	4·7	3·5	6·3	7·7	4·5
Very fine silt	1·2	5·6	2·3	5·5	3·2	6·3	3·9	3·3	3·8	6·4	2·0
Clay	21·7	23·2	29·0	27·0	26·5	38·1	36·5	36·6	39·4	44·8	42·3

In the case of black soils this factor cannot be made to account for the difference. The black soils, though commonly called "turf," are not appreciably richer, if at all, in organic matter or in silt fractions, than red soils of similar clay content. They usually contain appreciable amounts of carbonates whereas the red soils are usually slightly acid or neutral and, though they can be made to assume a good tilth, they are much more readily puddled, and furthermore in the process of mechanical analysis the removal of the clay fraction is more readily accomplished in the case of the black soils than in the case of red of similar or even lower clay content.

A comparison of the mechanical analyses of samples 3632 and 5531, and 5600 and 5344, would seem to show that these pairs of soils are similar in mechanical make-up. As will be seen in Table VI the physical properties are, however, widely different.

The fundamental difference between the grey and the black soils on the one hand and the red on the other has been noted by Vipond(10).

"The real difference between Groups 2 and 3 (*i.e.* between the grey and the red soils) is not a matter of colour, this being only an accompaniment of a certain condition. Whereas the typical grey soils break down to a thin mud on standing in water, and on being handled in water are readily reduced to a condition of partial deflocculation, the typical red soil breaks down slowly and reluctantly when worked in water, and does not at any time form a thin mud."

Vipond considered that the iron oxide (hydroxides) in the soil was responsible for this difference in the behaviour of the soils and took as a compensating standard the iron oxide, soluble in hydrochloric acid, contained in the clay fraction. The writer and Smit have discussed this question elsewhere(5). Not only do the ferric hydroxides have a flocculating effect on the clay, but a considerable portion of the so-called clay consists of ferric hydroxides or oxide which has not the properties of true clay.

It must be remembered that the data here presented are the results of laboratory experiments on laboratory prepared soil samples and that they cannot be regarded as reflecting absolutely the properties of the soils in the field. We believe, however, that they are comparable with one another and that similar data for the field soils will show similar relations and anomalies.

We believe that any difference there may be in the structure of red and of black soils of similar texture[1] does not satisfactorily account for the difference in physical properties, but at present hold the view that the portions of the soils classed by mechanical analysis as "clay" are fundamentally different in properties. Comber(1) has suggested that the fundamental difference between "fat" and "lean" clays is due to the higher proportion in the former of emulsoid surface to suspensoid core. It is possible that in the case of the black soils we have to deal chiefly

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[1] Most American writers term the field condition of a soil its "structure" and call the ultimate mechanical composition "texture." Hall uses these terms in exactly the opposite sense (2) and, judging from the context, Russell (9) does so also. We propose to follow the latter authorities as we consider that their use of the terms is more in keeping with the plain meaning of the words.

with kaolin and similar bodies which exhibit this emulsoid surface to a high degree while in the case of the red soils we have a large proportion of iron hydroxides which exhibit suspensoid properties only.

Robinson(8) holds the view that the soil colloids, the so-called ultra clay, exhibit practically the same specific water absorption no matter the class of soil from which they are obtained, but cites some exceptions including two which are stated to be rather unusual soils noted for their colloidal properties when wet. Now our Transvaal "black turf" soils are very unusual and exhibit colloidal properties to a marked degree.

It is possible, of course, that the difference between the red and the black soils discussed in this paper may be due to the presence in the latter of a larger proportion of colloids rather than to a fundamental difference in the nature of the colloids. It would seem, however, that the peculiar properties of the red soils are best explained by the view that the colloidal iron hydroxides have a strong coagulating effect on the colloidal clay. Further work on this subject is contemplated.

V. THE RELATION BETWEEN THE CLAY CONTENT
AND THE SPECIFIC GRAVITY.

According to Keen and Raczkowski both the apparent and the true specific gravity decrease with increasing clay. Our work demonstrated that this is only true for soils of the same or similar type and that specific gravity whether true or apparent is dependent to a large extent on the origin of the soil. We have determined true specific gravity both by the method of Keen and Raczkowski and by the bottle method: these two series differ considerably and there is no doubt that the former method gives low results, as has been pointed out by the originators. Our determinations show that apparent specific gravity and true specific gravity, whether determined by the bottle method or by the method of Keen and Raczkowski, does decrease with increasing clay content in a very general way provided only soils of similar origin are compared. As soils of similar origin have, in the Transvaal at least, similar texture it is not easy to correlate specific gravity with clay content.

In the following table the results are averaged and arranged as before.

Starting with the soils containing least clay the apparent specific gravity decreases with increasing clay content until the group averaging 17·3 per cent. of clay is reached, then there is a sudden rise followed by a steady decrease to the group containing most clay. This rise is co-incident with the inclusion of red soils derived in part from magnetite. The variations in true specific gravity follow somewhat similar lines but

are not as regular. If the results for heavy red, grey, and black soils be compared the fundamental difference between these classes is again apparent, as reference to Table IX will show. In this table the results for sandy soils grouped in two classes are also given. Those derived from granite have a higher apparent specific gravity than those derived from sandstones though the true specific gravity is the same for each group. It is evident that the granite soils can be more closely packed than the sandstone soils.

Table VIII.

Clay %	Apparent specific gravity	True specific gravity	
		(a) By bottle	(b) Keen and Raczkowski
4·5	1·62	2·65	2·35
7·4	1·48	2·65	2·30
12·7	1·43	2·64	2·27
17·3	1·40	2·71	2·27
21·7	1·47	2·82	2·38
27·4	1·44	2·87	2·41
32·9	1·38	2·95	2·41
37·5	1·33	2·74	2·30
42·9	1·30	2·72	2·23

Table IX.

Soil group	Clay	Apparent specific gravity	True specific gravity	
			(a) Bottle	(b) Keen and Raczkowski
Sandy soils from granite ...	5·8	1·61	2·65	2·31
Sandy soils from sandstone ...	6·8	1·45	2·65	2·32
Red soils containing magnetite	30·2	1·48	3·02	2·51
Grey soils	28·9	1·37	2·63	2·24
Red soils derived from diabase	36·1	1·27	2·79	2·33
Black turf soils	37·3	1·33	2·65	2·18

Nolte[6] has published some determinations of the specific gravity of various mechanical soil fractions and draws attention to the marked difference between that of the clay fraction and that of the other fractions, which latter were found to be fairly constant.

One would conclude, therefore, that the higher the percentage of clay the lower would be the specific gravity.

Judging from the samples examined we might say that true specific gravity is fairly constant for all types of soil with the exception of the heavier red ones.

The results are summarised below (Table X).

The specific gravity of about 71 per cent. of the soils lay between 2·60 and 2·70 and the average for these was 2·65; 12 of the remaining 14 are heavy red soils.

We consider that on the evidence at present at our disposal we are justified in drawing the conclusion that no relationship exists between the clay content of a soil and its true specific gravity, but that in a general way apparent specific gravity decreases with increasing clay content. In this connection, however, the relatively high apparent specific gravity of black turf soils is noteworthy.

Table X.

Specific gravity	No. of soils	Percentage of soils examined
2·60–2·65	17	35·4
2·65–2·70	17	35·4
2·70–2·75	2	4·2
2·75–2·80	2	4·2
2·80–2·85	4	8·3
2·85–2·90	2	4·2
2·90–2·95	0	0
2·95–3·00	1	2·1
Over 3·00	3	6·2

VI. RELATION BETWEEN VOLUME EXPANSION AND CLAY CONTENT.

In a general way the expansion per unit volume of the soil increases with increasing clay content but here again we are confronted with the strong difference in behaviour between red soils on the one hand and black and grey soils on the other. The expansion of the red soils was from 4 per cent. to 14 per cent. while black and grey soils containing the same range of clay expanded from just under 10 per cent. to over 45 per cent. of their original volume. All the results obtained are summarised and plotted as before (Fig. 5) but in this case it must be noted that in some of the groups extremely divergent values, such as 6·6 per cent. and 43·7 per cent. in the group averaging 37·5 per cent. of clay, are included in the average. This is due to black soils having a high expansion and red soils having a low expansion falling in the same group. Averages obtained in this way have not the value that a mean of quantities of more or less the same order of magnitude would have.

The relation of volume expansion to soil type is noteworthy. Of the sandy soils, four derived from granite showed an average volume expansion of 3·7 per cent. between the limits 3·5 per cent. and 4·1 per cent., while seven derived from sandstone had an average expansion of 1·7 per cent. between the limits 0·4 per cent. and 3·5 per cent. The difference between the expansion of heavy red and heavy black and grey soils has already been noted. Red soils derived from diabase showed on the average a lower expansion than similar soils which owe their origin in part to magnetite, the range for the former being 3·5 per cent. to 6·7 per cent.,

clay ranging from 31 per cent. to 45 per cent., while for the latter expansion ranged from 4·1 per cent. to 13·9 per cent., for clay from 21 per cent. to 38 per cent.
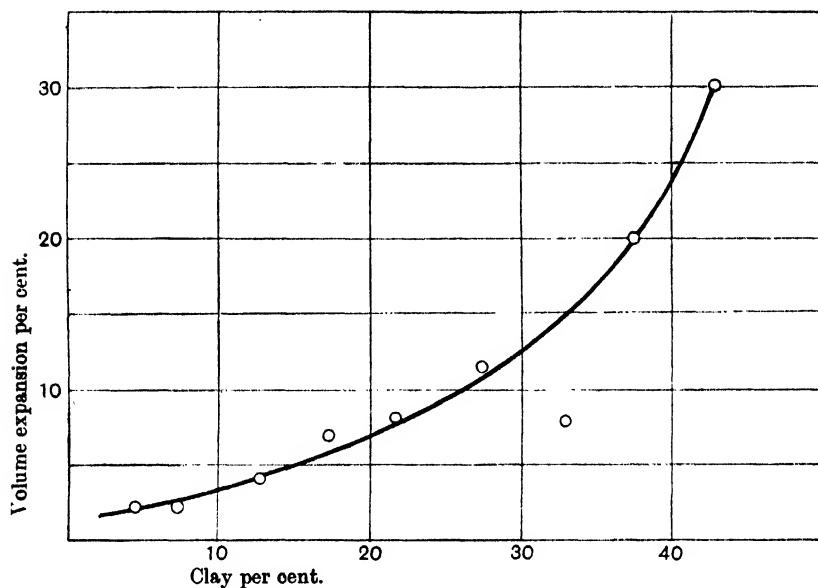

Fig. 5.

CONCLUSION.

In this paper an endeavour has been made to correlate certain physical properties of soils with their texture with a view to the interpretation of mechanical analysis. The method proposed by Keen and Raczkowski for the determination of the pore space, water capacity, apparent and true specific gravities, and volume expansion of soils has been followed.

It is shown that certain of these properties can be roughly correlated with the clay content of the soil but that the relationship is not sufficiently exact to be of much practical value.

Marked differences were found in the properties of certain soils of similar clay content but of different origin and appearance. It would appear that comparison of all soils, without discrimination, on the basis of mechanical make-up, would lead to utterly erroneous conclusions. The value of mechanical analysis lies chiefly, as has been pointed out by Robinson(7), in its relation to genetic classification. This is par-

ticularly true in a country like the Transvaal where the bulk of the soil is sedentary and large expanses of alluvial soil are unknown.

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ON THE MOISTURE RELATIONSHIPS
IN AN IDEAL SOIL.

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(With Four Text-figures.)

THE movement of water in soil, and the manner in which water is distributed over the particles and within the interstices, *i.e.* the dynamical and statical aspects respectively of moisture distribution, are of fundamental importance in soil science. The literature of the subject abounds with experimental determinations, but the difficulties of theoretical treatment are great, and it is only within recent years that any serious attempts have been made in this direction.

A mathematical study of the laws of water distribution in a natural soil, containing particles of all shapes and sizes, and colloidal material, is almost impossible; it is necessary to employ in such investigations a simplified conception, and to consider an "ideal soil," which is defined as a collection of spheres all having the same radius, packed together in a systematic way, and free from any colloidal material. When the spheres are packed as closely as possible, each one is surrounded by, and touches twelve others, and the pore space is very approximately 26 per cent. of the total volume; in the most open system of packing each sphere touches six others, and the pore space is approximately 47·5 per cent. Further, the percentage of pore space does not depend in any way on the radius chosen for the spheres.

A very complete study of the geometrical properties of the ideal soil was made by Slichter[1] in the elaborate joint work conducted with King on the movements of ground water.

In the ideal soil the water will be mainly present as annuli of wedge-shaped cross-section around the points of contact of the spheres. This conception of moisture distribution was introduced by Briggs[2] and has been developed by Buckingham[3], Gardner[4] and Wilsdon[5].

The purpose of the present paper is to draw attention to a necessary and simple consequence of the above hypothesis, which was not taken

[1] 19th Annual Report, *U.S. Geol. Survey*, 1899, pt. 2.
[2] *U.S. Bureau of Soils*, Bull. 10, 1897.   [3] *Ibid.* Bull. 38, 1907.
[4] *Soil Sci.* 1920, **9**, 191; 1920, **10**, 357; 103.
[5] *Mem. Dept. Agric. India* (Chem. Series), 1921, **6**, No. 3; *Proc. Punjab Engineering Congress*, 1923.

into account by Wilsdon, and which unfortunately invalidates a very attractive deduction that he arrived at from his analysis. It will be necessary in the first instance to summarise briefly the portion of his analysis that concerns us here.

The volume of water held around the point of contact of two equal spheres is that generated by the revolution of the meniscus about the point of contact. To a first approximation the meniscus is taken as the arc of a circle touching the two spheres. The volume (V) may then be expressed in terms of the radius (a) of the spheres and the angle (2θ) subtended at the centre of the soil particle by the radii from the point of contact of the spheres and the edge of the meniscus (Fig. 1):

$$V = \frac{8\pi a^3 \sin^4 \theta}{\cos^2 2\theta} \left[1 - \tan 2\theta \left(\frac{\pi}{2} - 2\theta \right) \right] \qquad \ldots\ldots(1).$$

$$\left[\text{Note.} \ \left(\frac{\pi}{2} - 2\theta \right) \text{ is in circular measure.} \right]$$

This value of V is then utilised in obtaining an expression for the moisture content (M) as a percentage on the weight of the soil as follows:

The number of particles per 100 grams of soil (n) is $n = \frac{100}{\frac{4}{3}\pi a^3 \rho}$, or taking ρ, the specific gravity of the soil, as 2·4,

$$n = \frac{31\cdot25}{\pi a^3} \qquad \ldots\ldots(2).$$

Each of these spheres touches a number of adjacent spheres, and the number of such contacts (C) depends on the degree of packing. For 100 grams of soil the number of such contacts is obviously $n\frac{C}{2}$ since each contact comes in twice. Thus the actual volume of liquid in 100 grams of soil is $Vn\frac{C}{2}$. For water the moisture content (M) per 100 grams of soil is $M = Vn\frac{C}{2}$, which from equations (1) and (2) becomes

$$M = 125C \frac{\sin^4 \theta}{\cos^2 2\theta} \left[1 - \tan 2\theta \left(\frac{\pi}{2} - 2\theta \right) \right] \qquad \ldots\ldots(3).$$

Turning now to the curved meniscus we have the familiar relation connecting pressure (P) and surface tension (T):

$$P = T \left(\frac{1}{r_1} - \frac{1}{r_2} \right) \qquad \ldots\ldots(4),$$

where r_1 and r_2 are the radii of curvature in the two principal directions and are given by AG and GD respectively in Fig. 1. When $r_1 = r_2$ the

pressure vanishes, and, neglecting the influence of gravity, this point should obviously represent the moisture-holding capacity of the soil for free water, *i.e.* the ideal soil should be incapable of holding water in excess of this amount.

Expressing r_1 and r_2 in terms of θ we have from the geometry of Fig. 1:

$$r_1 = \frac{a\,(1 - \cos 2\theta)}{\cos 2\theta}; \quad r_2 = \frac{a}{\cos 2\theta}(\sin 2\theta + \cos 2\theta - 1).$$

Equating these values and solving we have $\theta = 26° 33'$ nearly, *i.e.* when the angle 2θ in Fig. 1 is equal to $53° 6'$ the meniscus of the water wedge is a surface of no pressure, and the volume of water in the wedge has reached its maximum possible value.
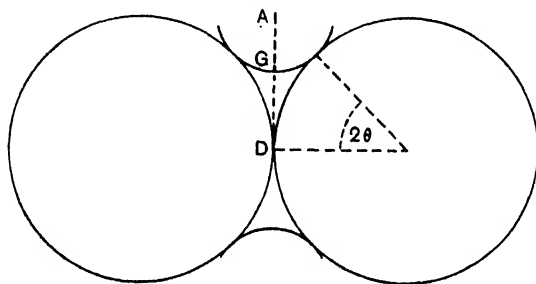

Fig. 1.

Hence, substituting this value of θ in equation (3) and assuming closest packing of the spheres ($C = 12$) we obtain the value $M = 23\cdot46$ as the maximum water-holding capacity (on a dry weight basis) of the ideal soil built up as above, and free from colloidal matter.

Having arrived at this value, Wilsdon points out that it is remarkably close to the value 21 occurring in the well-known empirical relationship of Briggs and Shantz[1]:

Moisture-holding capacity $= 4\cdot3 \times$ (Hygroscopic Coefficient) $+ 21$.

In the same paper Wilsdon gives both theoretical reasons and experimental data which lead to the view that the total amount of water held by the soil colloids, as distinct from the "free" or interstitial water, is represented by $4\cdot7 \times$ (Hygroscopic Coefficient).

The very considerable advance in our knowledge of water relationships which would be represented by the close agreement between the values 4·7 and 4·3, 23·46 and 21, is discounted by the fact that the derivation of the value $M = 23\cdot46$ cannot be substantiated. This value

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[1] *U.S. Bureau of Plant Industry*, Bull. 230, 1912.

was calculated for spheres in closest packing, in which case the pore space is 26 per cent. of the total volume. Now the maximum amount of water, calculated on the dry weight of the soil, that this soil could hold, if the pore space were *completely* full of water, would be only

$$\frac{26 \times 100}{74 \times 2\cdot4} = 14\cdot6 \text{ per cent.,}$$
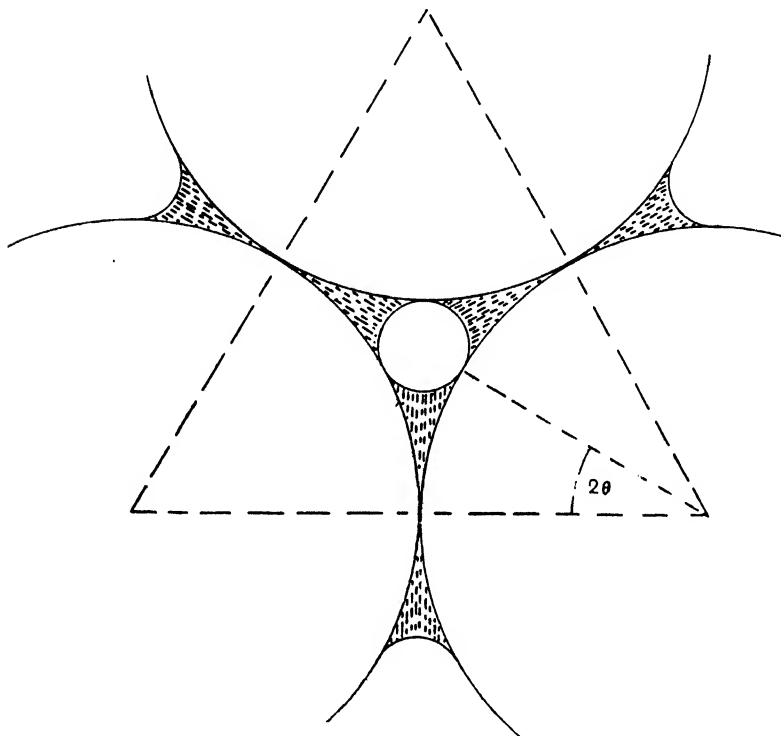
Fig. 2.

a value much less than 23·46 per cent. The discrepancy is larger even than the figures indicate, as the former is the maximum value for complete saturation, while the latter is developed on the explicit assumption that the individual water wedges remain distinct from one another, *i.e.* the pore space is not completely occupied by water.

The error has arisen because the single water wedge of Fig. 1 (for which Wilsdon's analysis still stands) has not been considered in its spacial relations to adjacent wedges. A consideration of one face of the regular tetrahedron (Fig. 2) obtained by joining the centres of the four spheres in closest packing (Fig. 3) will make this clear. In the plane of

this face the cross-section of the spheres and water wedges shows that the menisci of the latter will come into contact when the angle 2θ (cf. Fig. 1) is equal to 30°, at which point the moisture content, as calculated from equation (3), is only 3·55 per cent. Wilsdon's analysis cannot hold beyond this point, for if 2θ exceeds 30° the wedges would interpenetrate and a certain portion of the water would be included several times over.
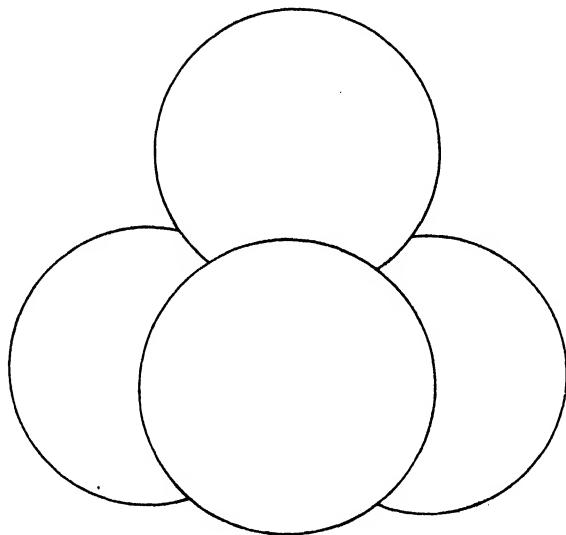
Fig. 3.

As Slichter[1] has shown, the section chosen in Fig. 2 is in the plane where the pore, regarded as approximately triangular in section, has its minimum cross-sectional area. Hence, at other points in the mass, the menisci will not yet be in contact when the value of M has reached 3·55 per cent. The analysis therefore breaks down slowly at first, but the discrepancy becomes rapidly more serious, because, as the following table from Wilsdon's data shows, M increases very rapidly as θ approaches its maximum value, and the error due to the separate inclusion of water held, as it were, in common by the wedges, also increases rapidly.

θ	M	θ	M
5·0	0·07	20·0	9·37
7·0	0·24	21·0	10·67
8·0	0·38	22·0	12·83
10·0	0·87	23·0	14·84
12·0	1·64	24·0	17·05
15·0	3·55	25·0	19·41
17·5	4·97	26·3	23·47

Loc. cit.

A further complication arises in the necessary adjustment in the curvature of the meniscus when adjacent water wedges meet, especially in the region where the pore has its maximum cross-sectional area, as r_1 and r_2 are no longer expressed by the simple relations given above.

For these reasons it is apparent that a system of spheres in closest packing is not suitable as a basis for the development of the calculations given above. If we consider, as an alternative, spheres in the most open or "cubical" packing, Fig. 4 shows that the menisci would not touch until $2\theta = 45°$, so that the above analysis would hold over a wider
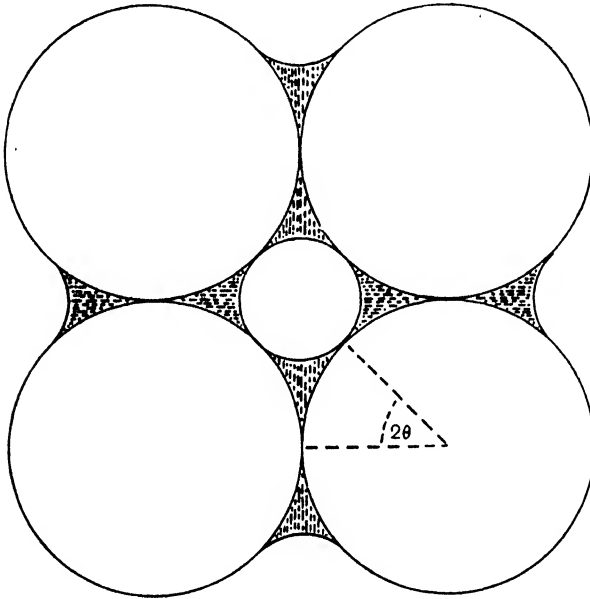
Fig. 4.

range. In calculating M for various values of θ from equation (3), however, it must be remembered that for cubical packing each sphere touches six others, so that in a given volume there are only half as many water wedges for cubical packing as for closest packing, and the values of M given in the above table must therefore be halved. When $2\theta = 45°$, M is approximately 7 per cent. for cubical packing. Beyond this value the analysis again breaks down. Quite apart from this error, due to interpenetration of the wedges, the value of M when θ equals its maximum value $26° 33'$, is no longer $23\cdot46$ per cent., but half this value; but it should be noted that as the pore space for cubical packing is $47\cdot5$ per cent. approximately, the value of M for complete saturation is $36\cdot2$ per

cent., so that if the theory can be modified to take account of the factors mentioned above, it may still be possible to obtain a theoretical significance for a value of M round about 21 per cent., for this system of packing.

Although the value 21 in the Briggs-Shantz equation may, and quite possibly does, represent the moisture-holding capacity of the soil for "free" water as distinct from that associated with the colloidal material, the value as yet rests only on an empirical basis. In this connection it should be pointed out that the interpretation of the constant 4·3 in the Briggs-Shantz equation as applicable to all soils, involves the assumption that the specific character of all soil colloids is the same. Wilsdon pointed out that this would be unlikely, and Hardy's[1] experimental study of this point has shown that the value of this "constant" does vary. While in all probability the variations in the specific nature of colloidal material from soil to soil are largely responsible for this, the conclusion is rendered less certain by our present inability to attach a theoretical significance to the remaining term, 21, in the Briggs-Shantz equation.

There is finally one other aspect of the problem that needs consideration—the distribution of water in an unbroken column of soil, saturated at the base. The simple theory of capillary rise involves the conclusion that, between the level of maximum rise and the ground water table, the soil will be completely saturated, whereas in actual practice the moisture content decreases with height.

Wilsdon's treatment of this problem appeared to give a very satisfactory solution, which agreed reasonably well with Buckingham's[2] experimental determinations, but the error discussed above was again involved. An attempt has therefore been made in the discussion below to give in a revised form the broad outlines of the probable mechanism of the rise of water in soil, it being understood that the influence of soil colloids in modifying these conclusions is not taken into account. We have already seen that for spheres in closest packing the menisci commence to come into contact when $\theta = 15°$ (or $M = 3·55$ per cent.), and that the pressure in any isolated water wedge would not become zero until $\theta = 26° 33'$. This surface pressure, when the water wedges reach the position shown in Fig. 2, will tend to draw the free water surfaces in this section of the triangular pore closer together, *i.e.* to increase the water content in, and eventually to fill the pore in this region. The net result of this process, which would take place at numerous points of

[1] *Journ. Agric. Sci.* **13**, 243, 340.   [2] *Loc. cit.*

contact throughout the mass of the soil, would be to draw water from adjacent regions, and by successive displacement eventually from the ground water itself. We thus arrive at a picture of the mechanism of water ascent in the ideal soil, which is a combination of the simple capillary tube hypothesis and the one originated by Briggs. In the first instance, water wedges are formed at the points of contact of the spheres; the peripheries of the wedges come into contact at the point where the triangular cross-section of the pore has its minimum area, and the pore in this region becomes full of water; this saturation will extend to the adjacent and wider parts of the pore, which will become completely filled, provided its dimensions are not too great. The whole process, in fact, is an illustration of the principle that the liquid tends to reduce its free surface, and hence its surface energy, to a minimum.

Equilibrium will be established, as in a capillary tube, when the weight of the liquid columns is sufficient to balance the surface tension.

In the case of a soil containing particles of diverse diameters there will be corresponding variations in the effective dimensions of the pore spaces. From our present point of view the soil column would be regarded as a collection of capillary tubes distributed over a certain range of effective diameters. Both the diameter range, and the number of tubes of any given diameter, would depend on the size-distribution curve[1] of the given soil. Thus for a soil of not too coarse a texture we should expect to find in successive planes above the ground-water level, (a) complete saturation, (b) complete saturation of smaller pores and incomplete saturation of the larger ones, and finally, (c) a region of incomplete and decreasing saturation; the moisture content would therefore diminish with height above the water level, as is known to be the case. By making some simple assumption for the relationship between the mechanical analysis of the soil and the dimensions and number of the pore spaces, it should be possible to test the above suggestion to a first approximation.

[1] As developed by Oden, *Int. Mitt. Bodenk.* 1915, **5**, 257; *Proc. Faraday Soc.* 1922, **17**, 327.

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COLLOIDAL PROPERTIES OF WINTER WHEAT PLANTS IN RELATION TO FROST RESISTANCE.

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(With Plate I and Two Text-figures.)

INTRODUCTION.

IN an earlier paper in this *Journal*(10) the author reported that wheat plants, in the winter-hardened condition, retained their water content with great force. For example, it was found impossible to express more than 2 to 3 c.c. of juice from 100 gm. of leaves, collected December 9th, 1920, under a pressure of 400 atmospheres. The significance of the property thus discovered lies in the fact that the first effect of freezing plant tissues is the withdrawal of water from the cells to form ice in the intercellular spaces. From the standpoint of frost resistance, forces opposing this desiccation must therefore be of great importance.

The osmotic pressure of the vacuolar sap no doubt contributes to the water-retaining powers of the cells, but as a complete explanation it is quite inadequate. In hardy varieties of winter wheat, the osmotic pressure of the sap has not been found to exceed 32·2 atmospheres, corresponding to a freezing-point depression of 2·68° C., whereas these varieties will resist temperatures of − 40° C.

The existence of much greater forces in wheat leaf tissues was shown by the difficulty of expressing their sap, and it was suggested, in the above-mentioned paper, that the principal force concerned was that of imbibition. The well-known hydrophilic properties of the colloidal pentosans and their rôle in the economy of certain drought-hardy plants, led to the conjecture that these substances probably accumulated in the cells during the hardening process induced by falling temperatures in late fall, and contributed largely to the formation of a protoplasmic gel of high imbibitional powers.

Since the preparation of the above paper, important publications by Rosa(13, 14) and Hooker(7) have appeared. These authors found that
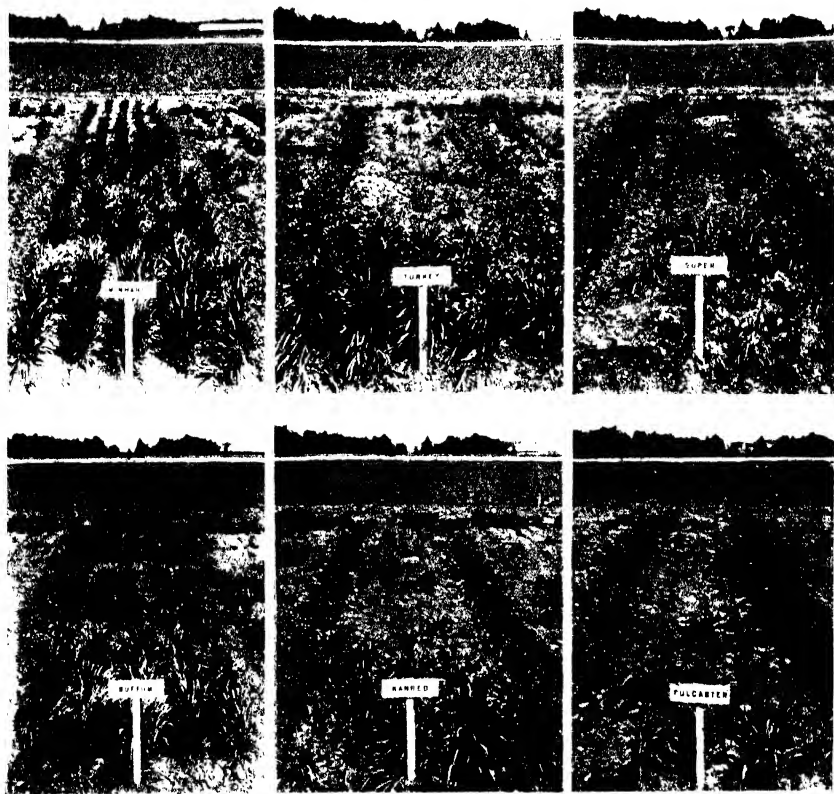
Winter survival of wheat varieties used, 1921–22. Photo, May 15th, 1922.

pentosans accumulate in the cabbage and other vegetables during the "hardening off" process, and that this accumulation may be brought about either by exposure to cold or by deprivation of moisture to the roots. Rosa also used the dilatometer freezing method of Foote and Saxton(4) to determine the quantity of "free" and "unfree" water in the tissue. This is probably more accurate than the earlier method used by Müller-Thurgau(9) for the same purpose. At any given temperature below freezing-point, Rosa found that the quantity of water separating in the form of ice was greater in the case of unhardened tissue. This amounts to an indirect measurement of the sum of the osmotic and imbibition pressures of the plant cells.

Further investigations on the nature of frost resistance in winter wheat have been carried on by the author during the seasons of 1921–22 and 1922–23, at St Paul, Minnesota, and Edmonton, Alberta. These are reported in part in the following pages, the data presented at this time having special reference to the colloidal properties of the tissues. The author has kept in view the possibility of developing a reliable "measuring stick" by which the plant breeder may estimate accurately the hardiness of a new strain the first season, rather than be forced to wait upon the results of field experiments extending over a period of years.

MATERIALS INVESTIGATED.

The following six varieties of winter wheat were used, and are named here in the order of their winter hardiness: Minhardi, Buffum, Turkey (Minn. no. 1487), Kanred, Super, Fulcaster. From the standpoint of their behaviour at St Paul, Minnesota, the first two may be classed as hardy, the next two as semi-hardy, and the last two as non-hardy. A photo taken May 15th, 1922, showing their winter survival at St Paul that season, is reproduced in Plate I.

Most of the material used was grown in field plots under normal conditions, but some material was grown in the greenhouse for comparison. From the standpoint of frost resistance the greenhouse material is probably somewhat comparable to summer vegetative tissues. All plants collected were cut off level with the ground, but since they had in no case reached the jointing stage, the material consisted of leaf blades and sheaths. The field material was in most cases frozen when collected, and was carefully sorted over, either in the field or in a cold room, for removal of dead tissue.

The fluid expressed from the tissues, with or without previous grinding, has been used a great deal in this study. For this the term

"press-juice" has been adopted, as being convenient and brief. This fluid, especially after grinding the tissues, cannot be regarded as merely vacuolar sap, so that the latter term appears unsuitable.

THE MEASUREMENT OF IMBIBITION PRESSURE.

The circumstances which first led to the observation of the high imbibition pressures of hardened wheat leaf tissues, namely, the difficulty of expressing the sap therefrom, suggested a method for its quantitative measurement. Reinke(12) used such a method in determining the imbibition pressure of *Laminaria*. He placed discs of this plant in a metal cylinder, and subjected them to heavy pressure from a piston perforated with many small holes, through which the expressed fluid passed. The imbibition pressure of the tissue, at any given moisture content, would then be equal to that pressure which just failed to express any further fluid. This method was modified in the present investigation to the extent that the piston was not perforated, but fitted loosely enough to allow the press-juice to pass around it. Fifty gram samples of fresh leaves were placed in a steel press-bowl about 3 inches in diameter, and pressure applied in progressive steps, the fluid expressed being allowed to drain into a graduated cylinder. This was facilitated by arranging the hydraulic press used in such a way that the press-bowl drained at an angle of depression of about 10°. A uniform practice was adopted in letting the press-bowl drain for two and a half minutes each time the pressure was increased, before reading the quantity of press-juice in the graduate.

IMBIBITION PRESSURE AND RELATIVE HARDINESS.

The quantities of juice obtained by the method outlined, from hardened leaves collected January 21st, 1922, and unhardened leaves collected May 17th, 1922, are shown graphically in the upper parts of Figs. 1 and 2. Since the imbibition pressure of a gel is not measured by the quantity of fluid expressed from it, but by the final concentration of the disperse phase at any given pressure, or in other words by the percentage hydration of its dry substance, this value has been calculated and plotted in the lower parts of the same figures. The value shown for hydration at any pressure is simply the residual water content at that pressure, expressed as a percentage of the dry matter.

The resistance to this pressure method of desiccation, of plants collected in the winter-hardened condition, is greatest in the hardy varieties, least in those which are non-hardy, and intermediate in those which are semi-hardy. A perfectly regular relation between hardiness

and the volume of press-juice obtained at various pressures is shown in the upper part of Fig. 1, thus constituting at once a simple and direct measurement of this quality. The quantity of press-juice is influenced, of course, by the initial moisture content of the tissues. Now, it has been found by several workers that there is a correlation between dry matter content and hardiness, a conclusion which is upheld by our own investigations. Since, therefore, the volume of press-juice is inversely proportional both to the hydrophilic colloid content of the cells and to the dry matter content, the pressure method used becomes a direct measure of the combined effect of these two factors, both of which appear to be related to winter hardiness.

In the lower part of Fig. 1 it will be observed that whereas relatively low values for initial hydration are associated with hardiness, and high values with a lack of hardiness, the order of magnitude is quickly reversed when pressure is applied, most of the curves intersecting at less than 25 atmospheres. As the pressure is increased, the hydration curves diverge again, with the higher values now characterising the hardy varieties. The irregular position of the hydration curve for Turkey is due to its unexpectedly low content of dry matter, for which no explanation can be suggested at present. The conclusion to be drawn from a study of Fig. 1 may be simply stated thus: the hardier the variety, the lower its moisture content, and the greater the force with which this is retained.   •

The foregoing conclusion is, however, valid only for leaf tissues which have been through the hardening process brought about by low temperatures or drying conditions such as obtain in late fall. This is proved by the results with summer tissues shown in Fig. 2. Here no relation can be detected between hardiness and volume of press-juice or imbibition pressure. There is, nevertheless, a surprising resistance to the pressing out of the tissue fluids from summer tissues. Part of this is merely a mechanical effect resulting from their coarser structure. No doubt the swelling pressure of the cell walls themselves enters in as a factor, in both summer and winter.

The specific relationships found in hardened tissues appear, however, to depend upon differences in the colloids within the cells. This is indicated by the almost complete loss of imbibitional properties when fresh tissue is killed by any agency, such as treatment with toluene, ether, or chloroform. An attempt was made by careful drying at low temperature to secure, if possible, air-dry tissue in which the colloidal properties were unimpaired, or at least not destroyed, but the effort proved fruitless.
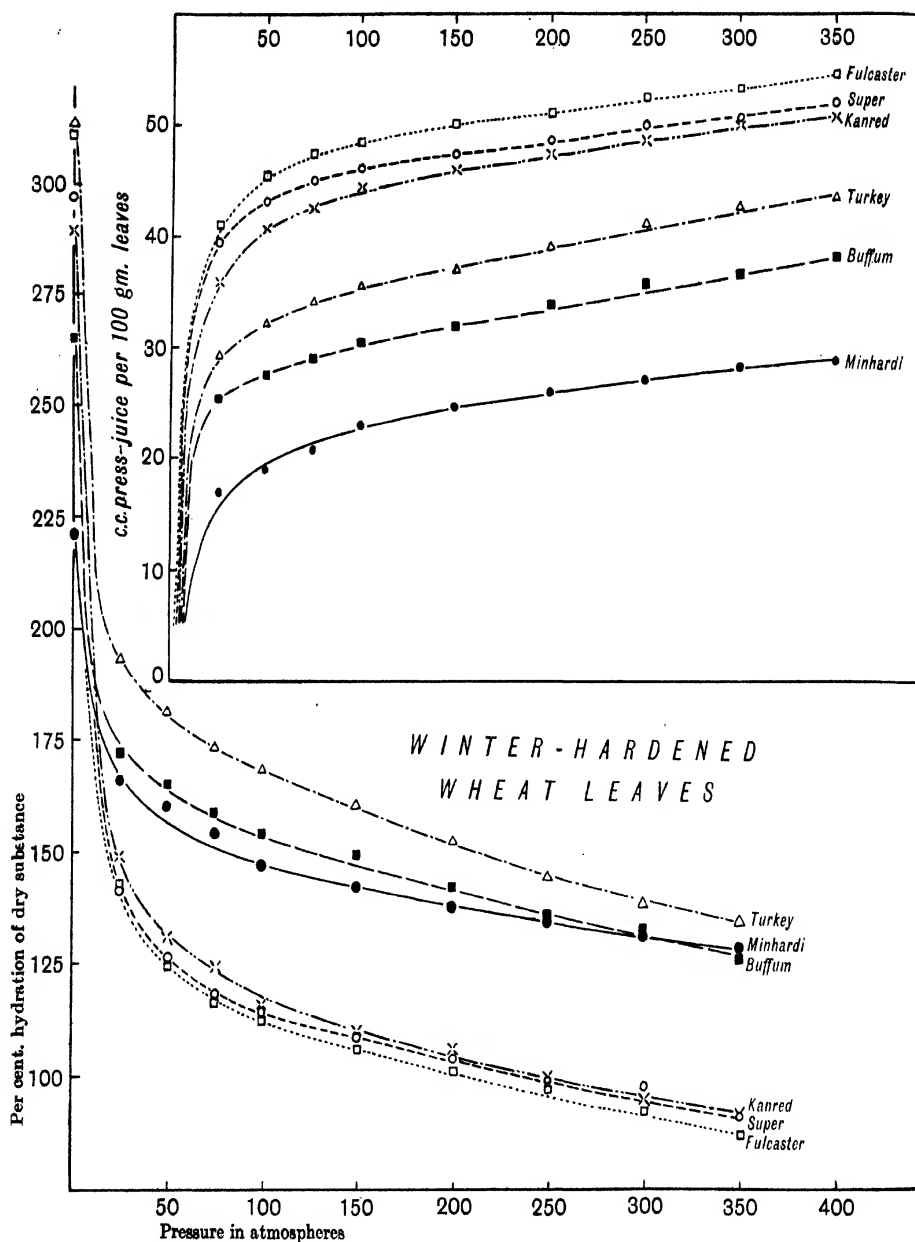
Fig. 1. Pressure dehydration curves of wheat leaves in the hardened condition, collected January 21st, 1922.
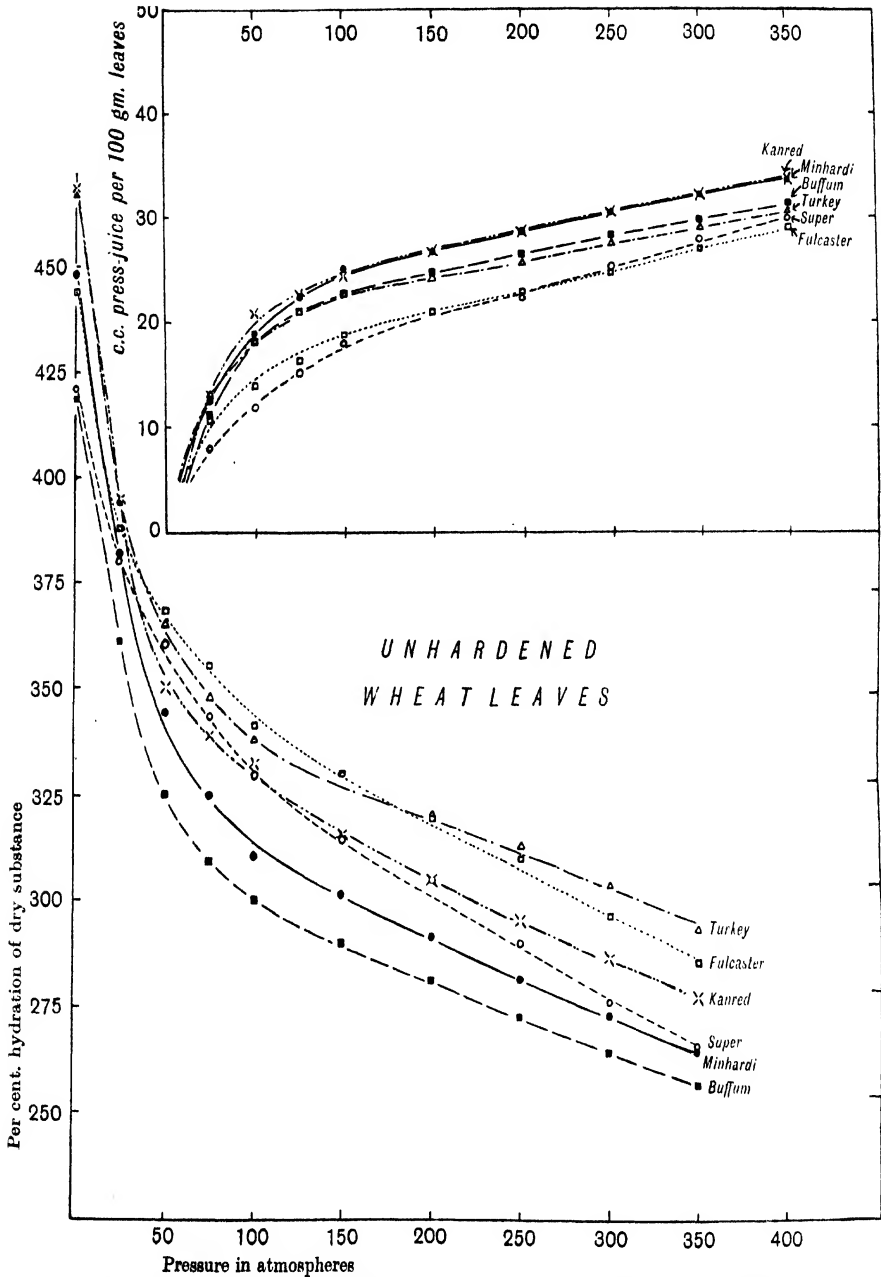
Fig. 2. Pressure dehydration curves of wheat leaves in the unhardened condition, collected May 17th, 1922.

Once the colloidal organisation of the cells is broken down, the characteristic differences in the water-retaining powers of hardy and non-hardy varieties is permanently lost.

A further comparison of Figs. 1 and 2 will reveal a fundamental difference between the types of the curves derived for hardened and unhardened tissues. The former partake of the nature of imbibition or adsorption curves, whereas the latter do not. The imbibition pressure of a gel increases very rapidly with dehydration, thus accounting for the characteristic logarithmic shape of an imbibition curve. The resemblance of the dehydration curve of hardened wheat leaves to an imbibition curve lends support to the view that the colloids which accumulate during the hardening process are largely concerned in the water relations of the cells.

It will be noted that quite appreciable volumes of press-juice were obtained from hardened wheat leaves in the winter of 1921–22, in contrast to the almost negligible volumes obtained the previous year, to which reference was made in the Introduction to this paper. This is, no doubt, explained by the difference in the two seasons. During the fall of 1920 the plots were entirely without snow protection, and the surface soil was frozen solid, a condition corresponding presumably with an extreme degree of hardening of the tissues, perhaps the complete gelation of the cell contents. In 1921, on the other hand, the plots were covered with snow before the advent of severe frost and remained in that condition continuously. The soil was saturated with moisture, and remained unfrozen until December. These milder conditions correspond with a much less development of imbibition pressure.

The conclusion of greatest practical importance to be drawn from this work on the imbibition pressure of wheat leaves, illustrated in Figs. 1 and 2 by two typical series of tests, is that the relative volume of press-juice obtained from the winter-hardened leaves of different varieties, under comparable conditions, is a fairly reliable measure of their relative hardiness. On theoretical grounds, the percentage hydration of the dry substance of the leaves at any pressure would seem a sounder basis of comparison; in practice, factors connected possibly with the structural elements of the tissues, rather than with the cell colloids, intervene to make it less dependable than the foregoing, simpler basis.

MOISTURE CONTENT.

It was pointed out by the author in a previous paper(10) that the moisture content was largely a function of the imbibitional powers of the cell colloids and the degree of previous exposure to modifying environmental factors. Beach and Allen(2) found that the twigs of hardier varieties of apple had on the average a slightly lower moisture content than those of the more tender varieties, but after a period of very cold weather, the situation was generally reversed, the twigs of the hardy varieties then containing most moisture. A parallel condition in plum varieties was reported by Strausbaugh(16), who showed that the moisture content of semi-hardy varieties dropped at low temperatures and increased in milder periods, whereas hardy varieties remained more constant, and at low temperatures had more moisture than the others.

Table I. *Moisture content of wheat leaves.*

A. *Collections of 1921–22, St Paul.*

Variety	Oct. 4th %	Nov. 8th %	Dec. 8th %	Jan. 21st %	May 17th %
Minhardi	78·3	74·4	77·6	68·8	81·8
Buffum	80·1	75·8	78·4	72·6	80·7
Turkey	80·7	77·1	79·3	75·8	82·4
Kanred	80·5	76·9	79·4	74·3	82·4
Super	81·8	76·8	81·0	74·8	80·0
Fulcaster	—	—	83·1	75·7	81·6

B. *Collections of 1922–23, Edmonton.*

	Nov. 18th	Nov. 25th	Dec. 2nd	Dec. 20th	Jan. 20th	Feb. 20th
Minhardi	68·9	68·6	69·7	69·7	67·9	68·8
Turkey	71·9	71·1	72·2	72·8	66·5	69·8
Fulcaster	72·7	71·7	73·1	73·6	70·8	73·7

C. *Variability of moisture content in winter of 1922–23, Edmonton.*

Variety	Mean	Standard deviation	Coefficient of variability
Minhardi	68·9	0·64	0·93 ± 0·18
Turkey	70·7	2·20	3·11 ± 0·61
Fulcaster	72·6	1·05	1·45 ± 0·28

The behaviour of winter wheat plants, as indicated by the data recorded in Table I, appears to be in line with that just noted. In this table the varieties are arranged in the order of their hardiness, and there is evident a considerable degree of correlation between this quality and dry matter content. This relationship is again specific for hardened tissue, and, as we have seen, it constitutes a contributory factor in determining the relative quantities of press-juice obtainable from these varieties. It is less marked in the collection of October 4th, 1921, when

the leaves were only slightly hardened, and completely absent in the collection of May 17th, 1922, when the plants were in a state of active growth. For the moisture content found in the six collections made at Edmonton, in 1922–23, while the plants were in the hardened condition, the coefficient of variability has been calculated. Necessarily with such a small number of observations, the probable error is large, and the unexplained drop in the moisture content of Turkey on January 20th greatly increases the apparent variability of this variety. But in view of the observations of Beach and Allen and Strausbaugh, with woody plants, it is very suggestive to find in wheat also the hardiest variety showing the least variability.

Recent researches have given a clue to the nature of this behaviour. Bancroft[1] cites the observation by Cartledge that gelatin gels prepared with different moisture contents appear to have definite structures. When dried to a uniform water content of 4 per cent., then placed in water, they swell rapidly to the percentage moisture at which they were originally prepared, but after that extremely slowly. Gortner and Hoffman[5] have further investigated this phenomenon, and secured additional evidence of a gel structure which modifies hydration relations. It may easily be inferred, therefore, that the resistance to extreme hydration as well as desiccation, shown by the hardened tissues of hardy varieties, is simply another expression of their highly colloidal nature.

CELL COLLOIDS IN PRESS-JUICE.

Since the imbibition pressure of winter-hardened wheat leaves has been found to depend on the colloids of the living cell, a study of these constituents becomes of great importance. Such a study involves considerable technical difficulty, owing to the extreme lability of the protoplasmic colloids concerned, and the necessity of extracting them from the fresh tissue, if possible in an unchanged condition. In the present investigation, the leaves from the open were usually frozen when collected. They were finely ground and pressed out as rapidly as possible, the precaution being taken to chill previous to use the meat grinder and press-bowl employed. The press-juice was centrifuged for the removal of suspended solid particles, in tubes jacketed with ice water, and kept cold till the completion of the work.

It may be assumed that the fluid thus obtained from finely ground material is a mixture of cell sap and cell colloids in somewhat the proportions in which they occur in the cells. Therefore, the properties of this

fluid should represent in some degree the properties of the cell contents. From the point of view of the colloidal constituents in their relation to winter hardiness, it is of particular interest to look for evidence of imbibitional properties.

The details have already been published(11) of a method depending on the imbibitional properties of hydrophilic colloids for the estimation of the relative concentration of these substances in expressed plant tissue fluids. Briefly, the procedure is as follows:

The freezing-point depression of the freshly expressed plant juice is first obtained. Then, having determined the total solids in the juice by the refractometric method proposed by Gortner and Hoffman(6), a fresh portion containing 10 gm. of water is weighed out. To this is added 3·4224 gm. of pulverised sucrose, a quantity just sufficient to make a molar solution in the total water present. The freezing-point depression is again determined, and is usually found to have increased more than the theoretical amount (2·085°, allowing for the formation of sucrose hexahydrate). It is assumed that the magnitude of the excess depression is a measure of the quantity of water held in such a way as to be unavailable for the solution of the sugar. This quantity is referred to as "bound" water. It represents the total water of hydration of all the substances in the press-juice, but it seems probable that in most cases the water bound by substances other than colloids is of minor importance. It should be added that under the experimental conditions observed, no increased depression of the freezing-point which could be attributed to invertase action has been found, in a somewhat extended series of determinations on the same sample.

The application of this method to our series of winter wheats, and a few other materials, yielded the rather striking results shown in Table II. In this table the percentage total solids given was read directly by a refractometer. The values for viscosity, recorded in the next column, were determined by a viscosimeter of the Ostwald type, in a constant temperature bath at 25° C.; the figures are the number of seconds required for 3 c.c. to flow through a capillary tube, through which the same quantity of distilled water flowed in 204 seconds. The symbols used as headings for the next four columns have the following significance:

Δ. The freezing-point depression of the freshly expressed juice.

Δ_a. The freezing-point depression after the addition of the sugar.

Δ_s. This is equal to $\Delta_a - \Delta$, the actual additional depression due to the added sugar.

Δ_x. This is equal to $\Delta_s - 2·085$, the amount by which the depression

found on addition of the sugar is in excess of that expected on theoretical grounds.

The percentage bound water is calculated by the formula

$$\text{Bound water} = \frac{\Delta_x\,89\cdot2}{\Delta_s},$$

where 89·2 is the percentage of free water in a molar solution of sucrose. This is based on the assumption that 1 molecule of sucrose combines in solution with 6 molecules of water, the evidence for which has been discussed by Scatchard[15].

Table II. *The influence of hydrophilic colloids on the physical properties of plant press-juice, in relation to type, hardiness, and environmental conditions of plant.*

Material used	Total solids %	Viscosity (Water = 204) secs.	Δ	Δ_a	Δ_s	Δ_x	Bound water %
Winter Wheat Plants, collected Feb. 3rd–18th, 1922, from the open							
Hardy Varieties:							
Minhardi	16·4	398	1·741	4·226	2·485	0·400	14·4
Buffum	17·8	419	1·719	4·158	2·439	0·354	13·0
Semi-Hardy Varieties:							
Turkey	13·5	360	1·273	3·612	2·339	0·254	9·7
Kanred	13·5	336	1·461	3·753	2·292	0·207	8·1
Non-Hardy Varieties:							
Super	9·7	292	1·085	3·279	2·194	0·109	4·4
Fulcaster	10·3	295	1·202	3·394	2·192	0·107	4·3
Greenhouse Plants, collected Feb. 10th–16th, 1922							
Minhardi wheat	8·5	285	1·147	3·284	2·137	0·052	2·2
Super wheat	7·1	267	1·000	3·106	2·106	0·021	0·9
Bryophyllum	5·9	235	0·474	2·555	2·081	−0·004	0·0
Cereus	4·9	637	0·505	2·803	2·298	0·213	8·3
Solutions of gum arabic:							
1 %	1·0	309	0·005	2·147	2·142	0·057	2·37
3 „	3·0	487	0·013	2·186	2·173	0·088	3·61
5 „	5·0	684	0·025	2·221	2·196	0·111	4·50
7 „	7·0	932	0·034	2·254	2·220	0·135	5·42
10 „	10·0	1438	0·048	2·294	2·246	0·161	6·39

A pronounced correlation is at once apparent between the percentage bound water and the relative hardiness of varieties. The close relation of the bound water to the content of hydrophilic colloid is well illustrated by the properties of the leaf press-juice of *Cereus*, a cactus-like plant. Comparing this with the semi-hardy varieties of wheat, it will be seen that the percentage of total solids is nearly three times greater in the case of the wheats, but these show no corresponding superiority in capacity to bind water. The explanation of this is forthcoming on examination of the values for viscosity and Δ. These reveal a marked difference in the physical properties of the press-juice from these different

types of plants. The large proportion of colloidal material in the juice of *Cereus* accounts for its high viscosity, small depression of the freezing-point, and high percentage of bound water.

Final proof of an intimate connection between bound water and hydrophilic colloids was secured by the use of artificial sols prepared from distilled water and highly purified gum arabic. As shown in the table, the bound water in these sols increases regularly with concentration. When the percentage concentration is plotted against the percentage bound water, a curve of the logarithmic type is obtained, suggesting the imbibitional nature of the phenomenon. It cannot but be observed, however, how much less efficient in the capacity of binding water is the artificial sol as compared with the natural plant colloidal solution obtained from *Cereus*.

Bound water, hydrophilic colloid content, and relative hardiness are thus linked together in close relationship. But here again the correlation with hardiness is probably specific for hardened tissues. The data for Minhardi and Super collected from the greenhouse do show some difference, it is true, but the values are too small to have much significance.

The influence of sugars on the percentage of bound water in press-juice cannot be entirely neglected. Due allowance is of course made for the hydration of the sugar added in the course of the determinations, but an initial concentration of 6 per cent. is often naturally present in the sap of hardy wheat varieties in the hardened condition. Calculated on the basis of sucrose, this would bind about 1·9 per cent. of water. But since sugar content is also correlated with hardiness (a point to be discussed in a later paper) the net result is merely that a method developed proves once again to be a measure of the additive effect of more than one factor for hardiness[1].

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[1] There seems little doubt, however, of the special applicability of the method to studies of colloidal properties. In a previous paper (10) the author pointed out a difficulty in satisfactorily accounting for variations in osmotic pressure on the basis of sugars and electrolytes only, and concluded "that sugars are probably not the only non-electrolytes which contribute to the osmotic values." The anomalous results secured at that time are readily explainable in the light of subsequent investigations of colloidal effects.

Anomalous results obtained by other workers may also, in some cases, be susceptible of explanation on this basis. For example, Dixon and Mason (3) found that sucrose in plant sap could be determined quite accurately by the change in freezing-point depression following inversion. They *boiled* the sap before use and added the necessary invertase solution. The boiling would of course coagulate the colloids, so that bound water would not come in question. On the other hand, Mason (8) later tried *fresh* sap, depending on the invertase naturally present, and storing the sap at temperatures suitable for enzyme

SUMMARY.

1. Certain colloidal properties of winter wheat plants have been found to be closely related to frost resistance, and provide indices of hardiness which may have practical application in the breeding and selection of hardy varieties.

2. The imbibition pressure of fresh leaves, in the winter-hardened condition, was found to be in most cases directly related to hardiness.

3. The volume of press-juice obtained per 100 gm. of hardened leaves was inversely proportional to the hardiness of a variety. This volume is largely determined by two hardiness factors, the dry matter content and imbibition pressure of the leaves.

4. When unhardened leaves were used, no relation could be found between imbibition pressure or volume of press-juice and hardiness.

5. The imbibition pressure of hardened leaves appears to depend on the physical state of the cell colloids characteristic of living tissues, since this property was lost when the tissues were killed.

6. The moisture content of hardened tissues tends to be inversely proportional to hardiness. There is some evidence that in hardy varieties it fluctuates less with changes in weather conditions, and a possible explanation of this as a colloidal property is suggested.

7. The quantity of hydrophilic colloids contained in the press-juice, as measured by an effect on the activity of water, was found to be directly proportional to hardiness.

action. He found inversion apparently incomplete, but reported that coagulation of the colloids occurred during storage, and suggested that the enzyme might have been inactivated by adsorption. The coagulation of the colloids would, however, have released bound water, thus diluting the solution and providing an explanation at least as satisfactory as the foregoing for the freezing-point depression being less than that expected.

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THE RAPID DETERMINATION OF AVAILABLE PHOSPHATE IN SOIL BY THE COERULEO-MOLYBDATE REACTION OF DENIGÈS.

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NUMEROUS measurements have been made of the phosphate content of soil extracts and attempts made to correlate the results with the behaviour of various crops. For purposes of extraction 1 per cent. citric acid has been used very extensively and to a lesser extent a saturated aqueous solution of carbon dioxide. Arrhenius (1922) has pointed out the desirability of carrying out the extraction at the pH value of the soil under examination for it is known that phosphates are more readily soluble in acid than in neutral or alkaline liquids.

A series of soil samples of various pH values was obtained in the course of an investigation into the relation of soil reaction to plant distribution. Work on sea-water led the writer to employ the coeruleo-molybdate method of Denigès (1921), as slightly modified by Florentin (1921) for phosphate estimations and it was found that this was suitable for the estimation of the phosphate yielded by soil at its own pH value.

The method consists in making a colorimetric comparison of a standard phosphate solution with an aliquot part of the diluted extract after adding 2 c.c. of reagent A, followed by five drops of reagent B to 100 c.c. of each. The mixing is made in a stoppered cylinder, after which the solutions are poured into 100 c.c. Hehner cylinders, and liquid is allowed to run out of one cylinder till a match is obtained. With most soils the blue colour is too faint for the Duboscq colorimeter to be of any use on account of its relatively short tubes.

Reagent A consists of a mixture of 100 c.c. 10 per cent. ammonium molybdate with 300 c.c. of 50 per cent. (by volume) sulphuric acid, which is stored in the dark. Reagent B is stannous chloride solution, freshly prepared each day, using 0·1 gm. of tin, one drop of 4 per cent. copper sulphate and 2 c.c. of pure hydrochloric acid. The reaction is hastened by warming, and finally the mixture is made up to 10 c.c.

These reagents when added to solutions of phosphates give a blue colour, which reaches its maximum intensity in about five minutes.

For the determinations 10 gm. of air-dried soil, passed through a sieve with 100 meshes to the inch, were shaken for 3–4 hours with 50 c.c. of conductivity water. Longer periods of agitation gave, in some cases, higher values, but more frequently they gave the same or even lower values, as shown by examples given further on. About 10 c.c. of the resulting liquid was centrifuged till clear, and 5 c.c. of the extract was then made up to 100 c.c. and after treatment as above described, the colour was compared with that given by a standard solution equivalent to 0·05, or in some cases, 0·5 mgm. of P_2O_5 per litre. The standard phosphate was preserved with toluene.

It was found that when reagent A had stood for some time a faint blue developed spontaneously, especially when exposed to light. This can be allowed for by means of a blank estimation with distilled water and has been found equivalent to a phosphate solution of 0·001 to 0·004 mgm. P_2O_5 per litre. Some soils give slightly coloured extracts, which impart a greenish tint to the blue liquid even when diluted twenty-fold as described, so that an exact match is impossible. To obviate this, drops of a dilute solution of Bismarck brown may be added to the standard, a very good match being thus obtained. The estimations made without Bismarck brown, where its use is advisable, are about 0·004 mgm. per litre too low, so this and the omission to allow for the blank on the reagents very nearly cancel out; accordingly the earlier estimations, in which these allowances were not made, are not seriously vitiated.

Using the proportions given, namely 10 gm. of soil with 50 c.c. of water, of which 5 c.c. are made up to 100 c.c. for testing, the result expressed in hundredths of a milligram of P_2O_5 per litre is numerically equal to the P_2O_5 in parts per million of the air-dried soil. Thus with 100 c.c. of diluted sample matched with 46 c.c. of 0·050 standard the concentration in milligrams per litre is 0·023, which becomes 0·019 (or 1·9 hundredths of a milligram) when corrected by the blank on the reagents. This is equal to 1·9 parts of P_2O_5 per million of soil.

In order to ascertain whether the constituents of soil extracts interfered in any way with the phosphate estimations, three direct tests were made by adding, to 5 c.c. of soil extract, 5 c.c. of standard phosphate containing 0·5 mgm. per litre. When diluted as usual to 100 c.c. the added phosphate enriches the solution so as to raise its phosphate concentration by 0·025 mgm. per litre. The enriched solutions were then analysed, also the original extracts with results as follows.

Table I.

Phosphate added to enrich solution mgm. P_2O_5 per litre	P_2O_5 in diluted extract mgm. per litre	pH of soil extract	Added phosphate found
0·025	0·000	6·0	0·0245
0·025	0·006	7·8	0·0240
0·025	0·025	7·4	0·0243

It so happened that none of the three solutions required the addition of Bismarck brown, viz. they gave clear blue tints. Had they done otherwise the agreement might not have been so good.

Further evidence of the freedom from error in the estimation due to the presence of various salts is afforded by the agreement between the analyses of the phosphate content of sea-water carried out by the writer (1923), using the coeruleo-molybdate method and by Matthews and the Government Chemist, London, using the colorimetric method of Pouget and Chouchak.

Since, in most cases, 5 c.c. of the one to five soil extract were made up to 100 c.c. for colorimetric estimation, the result in milligrams per litre should be multiplied by 20 to arrive at the concentration in the extract; thus, 0·019 mgm. per litre, as measured, is equivalent to 0·38 mgm. per litre in the undiluted extract. Again, the smallest concentration of phosphate that can be detected by the reaction is about 0·001–0·002 mgm. per litre, which, using the twenty-fold dilution, is equivalent to a concentration of 0·02–0·04 mgm. per litre in the extract. Work on natural waters (Atkins, 1923) has, however, shown that plankton algae can reduce the concentration to about 0·001 mgm., hence the limit 0·02 mgm., small as it is, may yet be surpassed without its being possible to assert that no phosphate is available. For still more delicate work a larger amount of soil might be used, and a dilution of less than twenty-fold.

Table II gives the amounts of phosphate, expressed as P_2O_5, found in a variety of soils, for which the pH values are also given. The sample serial numbers are the same as those used in an accompanying paper on the electrical conductivity of the soil extract.

It must be pointed out that these phosphate values reckoned as parts per million of dry soil are in reality conventional, not absolute, values, though extraction was made at the pH value of the soil. Inspection of the analytical results shows no correlation between the soil reaction and its yield of water soluble phosphate. The values obtained are for the most part under 2 p.p.m. unless enriched artificially. The figures found do not indicate any increase in solubility with increasing or decreasing acidity. It appears highly probable that increasing acidity does lead to increase

Table II.

Serial No.	Source of soil	Water soluble phosphate as P_2O_5 p.p.m. on air-dry soil, 1 : 5 extract		pH of extract
		Extracted 3–4 hours	Extraction time in days shown in brackets	
100.	Peat, S. Ireland	135	250 (20)	4·1
17.	Brown peat, Dartmoor	227	—	4·55
18.	Black peat, ‚ ,, ...	79	.—	4·85
1.	Skin of black soil, summit of Worcestershire Beacon, Malvern	1·0	0·9 (4)	4·65
2.	Decomposing archaean rock, from pit, Worcestershire Beacon	0·6	0·6 (4)	5·8
42.	Alluvial sand, Avondale, Co. Wicklow	0·0	—	5·6
15.	Alluvial deposit of 1922, R. Tigris, near Baghdad	1·1	—	7·6
	Arable land			
3.	Mangold field, Co. Cork	1·3	1·2 (4)	5·6
5.	Turnip field, Co. Cork	1·6	1·6 (4)	6·0
6.	Adjacent turnip field, treated with basic slag	3·3	1·0 (4)	5·8
97.	Potato field, dunged, Coombe Martin, Devon	3·5	—	7·7
101.	Garden soil, Cork, dunged	27	....	7·4
117.	Harpenden Field, Rothamsted ...	2·4	1·7 (4)	7·8
118.	Broadbalk, dunged plot, Rothamsted	20	15·1 (4)	7·8
119.	,, unmanured, ,,	0·4	0·4 (4)	7·8
120.	Leadon Court soil	0·6	2·3 (4)	7·4
	Pastures and waste lands			
8.	Pasture, Devon	0·5	1·1 (7)	7·5
7.	Subsoil of No. 8	0·6	0·5 (7)	8·0
53.	Permanent pasture, on level, Bovey Tracey, Devon	0·4	—	6·6
56.	Do. top of slope	0·2	—	6·5
57.	Do. bottom of slope	0·5	—	6·7
93.	Pasture, Coombe Martin, Devon ...	0·3	0·3 (29)	8·0
99.	Old pasture, Coombe Martin, no manure for 15 years	1·2	—	5·4
28.	Waste land, Bickleigh Vale, Dartmoor, Devon	0·3	2·5 (7)	6·0
36.	Do., furze burnt, Staddon Heights, Plymouth Sound	3·6	4·6 (7)	5·5
37.	Do. by pier Bovisand, Plymouth Sound, sheep rest	35·5	....	7·6
38.	Do. higher up	27·4	—	7·6
80.	Stony bank, limestone quarry, Oreston, Devon	2·0	0·4 (7)	7·7
81.	Grassy bank, Do.	1·0	1·4 (7)	8·0
82.	Stony bank, some grass, Fort Stamford, Devon	4·1	1·2 (7)	7·6
83.	Do. more stony	6·1	2·0 (7)	7·55
84.	,, ...	5·3	4·3 (7), 2·9 (11)	7·7
24.	Waste land, by sea, Plymouth Hoe, limestone	2·7	3·2 (11)	8·1
30.	Do. higher up	1·8	1·3 (7)	7·6
25.	Do. higher up again	3·8	1·9 (7)	7·8
31.	Pasture, Antony, Cornwall, with cattle	3·2	1·3 (7)	7·2
	Boulder clay			
39.	Ballyhoura Mts., Co. Cork, surface peat removed	0·0	0·1 (7)	5·05
40ᴀ.	Do. another site	0·0	—	5·4
40ʙ.	Do. blackish surface soil with remains of peat	1·6	—	5·25
41,	Do. another site	0·0	—	5·6

in phosphate solubility and further work on the point is in progress. The figures presented here do not, however, prove or disprove such a variation.

In this connection reference may be made to the recent researches of Burd (1918), Burd and Martin (1923), Hoagland, Martin and Stewart (1920), Russell and Prescott (1916), Shedd (1923), also of Hall and Vogel (1923), who have shown that the reversion of soluble phosphates added to soil progresses most rapidly in soils rich in iron. It seems probable, therefore, that the iron content is of great importance in regulating the yield to water during extraction. The decrease in phosphate shown, when water extraction is prolonged, is quite parallel to the phenomenon studied by Russell and Prescott with acid extraction[1].

The method of estimating phosphate devised by Denigès is obviously very much more rapid than the standard method of precipitation with ammonium molybdate and dissolving the precipitate in a standard alkali equivalent to $0 \cdot 5$ mgm. P_2O_5 per c.c., as given by the Association of Official Agricultural Chemists of the U.S.A., 2nd edition of *Methods of Analysis*, 1919. Prescott (1914) gives, for $N/10$ alkali, 1 c.c. equivalent to $0 \cdot 3004$ mgm. of P_2O_5; work is usually carried out on solutions containing 5–10 mgm. However, if $N/100$ alkali is used and a titration made to an accuracy of $0 \cdot 2$ c.c., which is very difficult with such a dilute alkali, the actual amount of P_2O_5 corresponding to the possible error is $0 \cdot 006$ mgm. With the colorimetric method, however, solutions as dilute as $0 \cdot 002$ mgm. per litre may be estimated on 100 c.c., the actual quantity involved being one-tenth of this, $0 \cdot 0002$ mgm.

The writer is indebted to Mr E. W. Fenton for a number of soil samples, also to Sir John Russell for those from Rothamsted and to various friends for miscellaneous samples.

Summary.

1. The coeruleo-molybdate method of Denigès affords a rapid means of estimating phosphates in such dilute solutions as are given by aqueous extracts of soils, 1 : 5, even when the extract is diluted twenty-fold.

2. High phosphate values were found for bog peats, but the majority of the soils studied gave to the extract phosphate corresponding to under two parts per million of phosphorus pentoxide. Dunged soil gave 20 p.p.m. or more.

[1] In this connection see also E. A. Fisher, *Trans. Faraday Soc.* **17** (1922), p. 312.

3. Extraction for 3–4 hours gives values as high as extraction for 4–7 days with ordinary soils of low phosphate content, but the phosphate of richer soils may undergo a reversion to an insoluble form during prolonged extraction.

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THE ELECTRICAL CONDUCTIVITY OF EXTRACTS FROM SOILS OF VARIOUS TYPES, AND ITS USE IN DETECTING INFERTILITY.

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(With One Text-figure.)

WHILE studying the relation between plant distribution and the hydrogen ion concentration of the soil it seemed of interest to attempt further to subdivide the various habitats according to the total soluble salt content. In particular it was thought that, in maritime situations blown spray might add appreciably to the soil salinity. This supposition was, however, found to be incorrect, except very near the sea, as apparently the rainfall is sufficient to wash out the sea salt. The investigation has brought to light very definite differences between the soils of diverse formations and positions. The method and the results with a number of soils form the subject of this communication.

The electrical conductivity of soil extracts has been studied by Beam and Freak (1914) and by Joseph (1921) in relation to the salt content as injuriously affecting the cotton crop. It was measured by Wilson (1915), together with the freezing-point, in studying the effect of heat upon soil. Joseph uses an extract of one of air-dried soil to five of water, after shaking and allowing to stand for one day.

The one to five proportion has been used largely of late for work on soils and Hoagland, Martin and Stewart (1920) have demonstrated that it gives, for light soils at any rate, a trustworthy picture of the composition of the soil solution, which has considerable value in comparisons.

In the present research soil taken from the top 6 inches was dried in air at room temperature. It was passed through a sieve of 100 meshes to the inch, and 10 gm. of soil, correct to within the first decimal place, were weighed out and stored till the determination could be made.

The sample was then placed in a bottle with 50 c.c. of conductivity water and shaken at intervals for three to four hours. This serves to bring into solution the more readily soluble salts. The mixture was examined at intervals up to about a fortnight or such lesser period as

sufficed to bring it approximately into equilibrium; this involves not only chemical, but biochemical, changes resulting in the production of carbon dioxide and consequent increase in calcium bicarbonate in some soils. This production of carbon dioxide was shown by the diminution in the pH value of the soil extract on standing after subsidence of the soil particles, the buffer action of which greatly reduces the observed change. That the increase in acidity was due to the production of carbon dioxide was demonstrated by agitation of the solution with air, whereby the gas was extracted and the original pH value recovered. It was noticed that in certain very uniform calcareous silts from Behar, India, these changes in the extracts took place much more rapidly in soil from the top 6 inches, than in those from 6–12 inches, and the latter again, though less markedly and less regularly, alter more rapidly than extracts from soils taken at 12–36 inches. This might be expected since bacteria are more numerous near the surface.

The examination consisted in centrifuging about 10 c.c. of the mixture and determining the electrical conductivity in the usual manner with a Kohlrausch bridge. For this purpose centrifuging is unnecessary, since a turbid extract, after depositing the larger particles as mud, gives the same conductivity as after centrifuging. However, the samples were in some cases used for hydrogen ion and for phosphate determinations, as described in an accompanying communication, in which the same serial sample numbers are used.

All measurements were made at $0°$ C., as the most convenient steady temperature. The cells were standardised with $N/100$ potassium chloride, which according to Kohlrausch has a conductivity 0·000,78 at $0°$ C. The cells used gave as constants 1·02 to 1·04, which for convenience and because the measurements did not justify greater accuracy were taken as 1·00. The error comes in partly in the measurement of conductivity and partly in the variation in the water content of the air-dried soils.

The possibility of error due to the solubility of the glass bottles used was examined and was found to be so small as to be negligible. Using pure distilled water in the cells the resistance was always greater than 10×10^4 ohms after standing in the glass bottles. With a cell of constant 0·102 the water which initially had a resistance of more than 10×10^4, corresponding to a conductivity of under 0·000,001,0 only increased to 0·000,002,7 in four days standing in the bottle. The constancy of the figures given by Nos. 39–41 up to seven days furnishes additional evidence on this point. All corks used were coated with paraffin wax.

The electrical conductivity of the extracts is shown in the table, for each sample after standing 3–4 hours and for many of them after standing, with frequent but not continuous shaking, for the number of days as shown in brackets after the figures. The pH values of the soils are also recorded. The behaviour of certain typical soils may readily be grasped by inspection of the accompanying figure. Most of these results have been omitted from the table.

Table I (*as revised and abbreviated*).

Serial No.	Source of soil sample	Conductivity at 0° C. $\times 10^6$		pH of extract
		Extraction 3–4 hours	Extraction time in days in brackets	
1.	Skin of blackish soil, over rock, summit of Worcestershire Beacon, Malvern	48	56 (4)	4·65
2.	Disintegrating archean rock from pit, same locality	53	52 (4)	5·8
26.	Earthy bank of meadow, Bickleigh Vale, Dartmoor	47	57 (7), 62 (12)	5·4
29.	Meadow, do. ...	35	57 (7), 61 (10)	5·8
28	Under clover sod, road, do.; Boulder Clay, Ballyhoura Mts., Co. Cork, skin of peat removed, 39–41	29	62 (7), 72 (10)	6·0
39.	Streamhill Glen, nursery	49	50 (7)	5·05
40A.	Do Compt. 13, red-brown soil ...	17	20 (7)	5·4
40B.	Do. blackish surface soil	24	26 (7)	5·25
41.	Do. Compt. 11	21*	19 (7)	5·6
5.	Turnip field, on hill, Co. Cork ...	110	106 (4)	6·0
3.	Mangold field, heavy soil, below No. 5	210	220 (4)	5·6
4.	Same field, plants poor	200	220 (4)	5·4
8.	Pasture, Devon	98	156 (4), 180 (9)	7·5
7.	Subsoil of No. 8	67	103 (4), 118 (7), 122 (9)	8·0
31.	Pasture, Cornwall, on Upper Devonian slates	89	250 (7), 260 (10)	7·2
32.	Adjacent field	63	176 (7), 196 (10)	6·9
117.	Harpenden field, July 1923... ...	59	111 (4), 156 (11)	7·8
119.	Broadbalk, unmanured plot, 1922 ...	66	115 (4), 154 (11)	7·8
118.	„ dunged plot, 1922 ...	102	208 (4), 261 (11)	7·8
120.	Leadon Court soil, 1923	87	161 (4), 196 (11)	7·6
82.	Rubble bank, Middle Devonian shale, underlying limestone	122	294 (7), 303 (11)	7·6
83.	Do.	145	294 (7), 308 (11)	7·55
84.	Do.	122	263 (7), 294 (11)	7·7
80.	Limestone rubble bank	116	244 (7), 270 (11)	7·7
81.	Steep grassy bank, same quarry ...	106	244 (7), 270 (11)	8·0
37.	Earthy bank, Bovisand Pier, splashed by sea	192	415 (7), 455 (10)	7·6
38.	Do. 10 ft. higher up, both on Staddon Grits (with slates)	190	400 (7), 425 (10)	7·6
18.	Dartmoor, black peat	370	377 (2)	4·85
17.	„ brown peat	500	526 (2)	4·55
100.	Bog peat, S. Ireland	666	—	4·1

* Fresh fractured surface of chip of glass from centrifuge tube appears to have raised the conductivity of this extract.

On viewing the table it is at once evident that the alkaline soils give high conductivity values, which increase still further on standing to values which are remarkably constant for each situation, considerably more so than were the initial values. High values, quickly attaining their maximum, are also given by peaty soils.

Times of extraction in days.
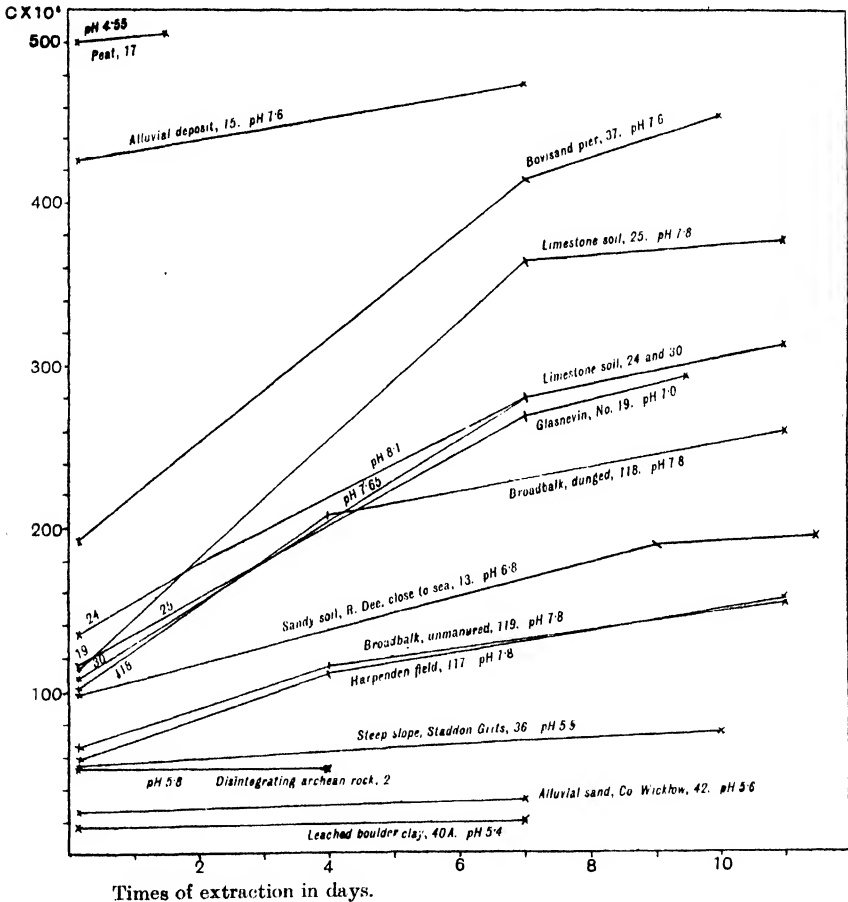
As a general rule acid soils give low results and a maximum is soon reached. Nos. 1, 2, 26, 29 may be instanced. It seems probable that the initial values depend to a considerable degree upon the extent to which the crop has depleted the soil of soluble materials, and this point requires further work to trace the seasonal changes. It is not suggested that the value of the soil is proportional to the quantity of salts it yields when

extracted with water, namely, at, or close to, its own hydrogen ion concentration, for excess of calcium bicarbonate, for instance, must be nearly valueless. It seems nevertheless to be true that a soil yielding a very dilute extract must be too poor in all necessary soluble mineral ingredients to be of agricultural value. This point may be illustrated by reference to Nos. 39–42. These poor soils were considered to be suitable for plantations. For the samples and information concerning them the writer is indebted to Mr A. C. Forbes of the Irish Forestry Department. "The soil consists chiefly of glacial drift on Old Red Sandstone. A thin covering of peat has been more or less removed for fuel purposes, leaving the surface almost entirely bare of vegetation. A few inches below the surface the soil becomes very compact, water resting on it throughout the greater part of the winter, but becoming very dry in summer. Iron pan is more or less universal. Trees planted on this soil make very little growth for several years."

As shown by the writer previously (1923) the percolation of acid peaty water would remove iron salts, which on penetrating into the less acid boulder clay would be precipitated, forming iron pan. It is noteworthy also that the water soluble phosphate in these soils, determined as described in an accompanying paper, is less than 0·1 part per million of air-dried soil. In spite of this phosphate in the form of basic slag showed no result, though kainit was beneficial; it appears that lack of potassium was the limiting factor rather than lack of phosphate. It appears that the acid water of the overlying peat had in course of time extracted almost everything soluble from the surface soil. When the roots penetrated deeper, however, whatever the boulder clay contained originally was at their disposal.

No. 42, according to Mr Forbes, "is taken from Avondale, Co. Wicklow, and is alluvial sand on the edge of a river which flows through old mine workings a few miles further up. For a number of years nothing grew on this soil of any kind, and trees planted in it died. It is now getting gradually covered with lichens, mosses and one or two grasses, chiefly *Molinia*. The soil is, however, practically barren." Here the acidity, pH 5·6, is not excessive. In view of the work of Miss Carpenter (1922) upon lead in Welsh rivers the soil was tested for lead, but none could be detected; it is, however, possible that flood waters may have contained enough to kill the trees, etc. The phosphate content was again found to be under 0·1 p.p.m. of P_2O_5, and a very low conductivity was observed. The small amount of this sandy soil which passes the 100-mesh sieve makes the observed conductivity rather too high in comparison with

other finer soils. Here, again, it appears that this thoroughly leached soil is too poor in soluble matter adequately to support vegetation.

The very infertile soils, Nos. 39–42, yield a 1 : 5 extract closely similar to the purest natural waters obtained from granitic or siliceous palaeozoic rocks, whereas the conductivity of the more fertile and calcareous soils approximates to that of streams in lowland regions. It is not, however, suggested that the water of the streams is similar to the extracts in its content of the necessary minor constituents such as nitrates, etc.

In conclusion the author desires to acknowledge his indebtedness to those among his friends who so kindly supplied soil and water samples.

SUMMARY.

1. The electrical conductivity of aqueous extracts of soils, in the proportion of one of soil to five of water, varies according to the time of extraction. In the more fertile soils extraction for three to four hours gives less than half as great a conductivity as is given in 4 to 11 days.

2. In peat the maximum value is reached almost at once, the extract having a high conductivity. The maximum value is also reached quickly in certain infertile soils, which give an extract of very low conductivity, closely similar to that of the purest upland streams.

3. A high electrical conductivity in the extract may only indicate the presence of an excess of salts, and does not necessarily indicate a good soil. It seems, however, that a rapid increase in conductivity as extraction is prolonged indicates increased solubility, partly through bacterial action and may be considered as a useful indication of fertility. A low conductivity, which remains low on continued extraction, denotes a soil so insoluble as to be unfertile.

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REMARKS AND OBSERVATIONS ON IMBIBITIONAL SOIL MOISTURE.

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(With Two Text-figures.)

In three earlier papers[1] the results of a study of the rate of evaporation of water from wool, sand, kaolin, ball clay, and various kinds of soil were described and discussed. It was shown that, except in those cases mentioned below, when the rate of evaporation is plotted against the water content (on a dry weight basis) the curve obtained is made up of three straight lines cutting one another sharply so that there are points on the curve at which there are sudden changes of direction. The factors determining the shape of the curves were discussed and it was suggested that these are four in number: the vapour pressure of the water present, the variable temperature of the drying mass and two others due to capillarity, viz. the diminishing rate of movement of water or vapour from the interior of the material to the surface as drying proceeds and diminution in area of evaporating surface as the larger capillary spaces become empty or as the water wedges between the soil grains get progressively smaller.

It was shown that with the materials mentioned the first sloping portion of the rate curves could be accurately expressed by the equation

$$-\frac{dw}{dt} - A = kw \qquad \dots\dots(1),$$

in which A is the intercept cut off from the vertical axis when the curve is extrapolated to $w = 0$, thus confirming the earlier experimental work of B. A. Keen[2] on sand and ignited soil.

It was further shown that with a deep clay subsoil containing 55·4 per cent. clay a similar type of curve was obtained but the portion under consideration exhibited considerable curvature. From the analogous

[1] This *Journal*, **13** (1923), p. 120; *Roy. Soc. Proc.* **103 A** (1923), pp. 139, 664.

[2] This *Journal*, **6** (1914), p. 456.

work of Keen it is probable that such curvature will be found to be characteristic of the evaporation curves of all soils containing more than small amounts of clay and the writer showed that this curved portion of the evaporation curve could be accurately expressed by an equation of the general type

$$-\frac{dw}{dt} - A + a\,(w-n)\left[-\frac{dw}{dt} - A\right] = kw \quad \ldots\ldots(2),$$

in which A, a and k are constants and $n =$ percentage water content at which the curvature ceased. This particular type of curvature appears to be found only in the evaporation curves of such materials as clay soils which are mixtures of colloidal and non-colloidal substances and has not been found in the cases of substances that are wholly colloidal, *e.g.* wool or ball clay, or wholly non-colloidal, *e.g.* sand or silt, and it was shown by the writer to be due to the simultaneous evaporation of imbibitional or "gel" water held by the colloid and of capillary or interstitial water held as water wedges between the soil grains. The former water evaporates at a practically constant rate, as was demonstrated in the case of swollen gelatin gels, while the latter evaporates at a rapidly diminishing rate, in accordance with equation (1); the actual observed rate is the resultant of these two and is represented by the curved portion of the rate curve. This imbibitional or "gel" water is associated in some way other than interstitially not only with the clay particles present but probably to an even greater degree with the gelatinous or colloidal coating surrounding the soil grains. Recent work by N. M. Comber[1] suggests that the gelatinous coating does not consist of pure clay particles but resembles in its peculiar protective action silicic acid and some other members of the so-called "emulsoid" group of colloids. Such a protective coating would give an "emulsoid" character to what would otherwise be a "suspensoid" system[2] and in the present paper will be discussed a possible explanation of the manner in which imbibitional water (as distinct from interstitial or capillary water) is held by the soil colloids.

The work of Green and Ampt[3] on the permeability of soils to air and water illustrates in a striking manner the difference in behaviour towards water of the colloidal and the non-colloidal constituents of a soil. The permeability (P) of a system of particles to a fluid is known to vary

[1] This *Journal*, 10 (1920), p. 425, 11 (1921), p. 450, 12 (1922), p. 372; *Journ. Soc. Chem. Ind.* 41 (1922), p. 77 T.

[2] For a further study of this type of "protection" see J. Loeb, *Journ. Gen. Physiol.* 5 (1923), p. 479.

[3] This *Journal*, 4 (1911–12), p. 1.

inversely as the viscosity (η) of the fluid used. Hence if two different fluids are used, *e.g.* air (a) and water (w), with the same system of particles $\dfrac{P_a \eta_a}{P_w \eta_w}$ should equal unity provided the permeability is unaffected by the fluid used. This ratio in the case of soils was found by Green and Ampt to be very much larger than unity and its value increased enormously as the colloid content of the soil increased. This was attributed by Green and Ampt to the imbibition of water by the clay particles and by the colloidal surface of the soil grains which would result in a restriction of the passages between the particles. A further consequencè would be that such a soil when allowed to drain to equilibrium under the influence of gravity will hold up more water than if no colloid were present.

In the case of some extreme types of organic soils such as moorlands and peats Crump[1] found that for any one type of such soil the water content under normal field conditions minus the water content in the air-dried condition at 15° C. bore an approximately constant ratio to the content of organic matter.

Table I. *Comparison of critical moisture contents and moisture equivalents of various soils.*

Soil	Percentage organic matter	Percentage clay fraction	Critical moisture content	Moisture equivalent
Heavy clay subsoil	13·6	55·4	21·0	55·1
Silty soil	7·1	11·5	14·8	31·0
Quartz sand ...	0·0	0·0	4·6	2·4
Garden soil ...	Considerable but undetermined	Not determined	24·0	24·0
Wool fabric ...	—	—	31·0	53·3

Few data so far have been published concerning the relation between water content and clay content but some light has been thrown on this matter by the writer by comparing the critical moisture contents, *i.e.* the moisture content at which the rate of evaporation begins to fall off from constancy, and the moisture equivalents of the same samples as determined by the standard method of Briggs and McLane[2]. The actual values are given in Table I[3]. It will be noticed that where no colloidal matter is present, *i.e.* with quartz sand, the moisture equivalent is less than the critical moisture content and a reference to Fig. 6 of a former

[1] *New Phytologist,* **12** (1913), p. 125.

[2] U.S. Dept. of Agric., Bur. of Soils, *Bul.* **45** (1907). Moisture equivalent is the term employed to denote the percentage of water retained by a soil when subjected to a constant centrifugal force sufficient in magnitude to remove the moisture from the larger capillary spaces.

[3] For the determination of moisture equivalents the writer is indebted to Dr A. F. Joseph, Government Chemist, Sudan.

paper[1] will indicate that the moisture equivalent lies somewhere on the first *sloping* portion of the rate of evaporation curve, indicating that the moisture had been removed from the larger capillary spaces leaving water present only in the smallest interstices. In the other cases given (except the garden soil) the moisture equivalent is very much larger than the critical moisture content. Some of the moisture equivalent is undoubtedly due to slight permeability as indicated by Green and Ampt's work but probably not very much since Briggs and McLane showed that with a Leonardtown loam increasing the time of centrifuging from 15 to 60 minutes decreased the moisture content by only about 2 per cent., while puddling a Cecil clay subsoil (containing 59·8 per cent. clay), increased the moisture equivalent from about 33 per cent. to about 39 per cent., this small increase being due to diminished permeability due to the puddling: the difference between the moisture equivalent and the critical moisture content of the heavy (Rothamsted) clay subsoil is about 34 per cent. From these data, slender though they are, it appears that when a wet soil is subjected for a considerable time to a centrifugal force some 1000 times that of gravity it will retain a large quantity of water, approximately equal to the difference between the moisture equivalent and the critical moisture content, in some way other than by capillarity. The striking difference between the results for sand and clay can hardly be explained in any other way: the water present in a soil is held in two different ways, part of it is present as capillary or interstitial water and part of it is imbibed, *i.e.* held in some way other than interstitially, by the clay particles and organic matter and by the colloidal coating on the soil grains.

Further Briggs and McLane showed that the moisture equivalent was due mainly to the clay and organic matter, the sand and silt having but slight influence on the amount of water retained. They give the approximate equation $ME = 0·13\,C + 0·62\,(D + E)$ in which D = clay content, E = content of organic matter and C = silt. This indicates that clay and organic matter are equally effective, weight for weight, in water retaining capacity. Their results of course include capillary water, which is different in amount with different soils, while in Crump's experiments this was to some extent excluded as the water content of the air dry soil was deducted from the amount found to be present in the field. On the other hand, the moisture equivalent has greater constancy than Crump's moisture content under normal field conditions. Probably a more accurate equation would be found to hold between "moisture equivalent

[1] E. A. Fisher. This *Journal, loc. cit.*

minus critical moisture content" and the content of clay and organic matter.

This imbibitional water can be studied from another point of view—a point of view that has proved particularly fruitful in another field of work, viz. in the study of the colloid behaviour of proteins. Donnan[1], in 1911, demonstrated that when a membrane separated two solutions of electrolytes, one of which contains an ion, *e.g.* a colloidal ion of a dye or of a soap, which cannot diffuse through the membrane, while all the other ions can diffuse through the membrane, there will be an unequal distribution of the diffusible ions on the opposite sides of the membrane. At equilibrium the products of the concentration of each pair of oppositely charged diffusible ions will be the same on both sides of the membrane. *E.g.* suppose a solution of a colloidal basic dye that can combine with acids is placed inside a collodion bag which is immersed in a dilute solution of HCl. The collodion membrane is permeable to all the ions and molecules except the undissociated dye and its cation. If $x =$ concentration of the H and Cl ions in the outside solution, $y =$ concentration of the free H and Cl ions of the hydrochloric acid inside the collodion bag and $z =$ concentration of Cl ions in combination with (or rather that belong to) the colloidal dye, then at equilibrium we should get

$$x^2 = y\,(y + z).$$

Such an unequal concentration of the crystalloidal ions must give rise to a difference in osmotic pressure on the two sides of the membrane because whereas the Donnan equilibrium described above depends on the fact that the product of the ionic concentrations must be the same on the two sides of the membrane, *i.e.* $x^2 = y\,(y + z)$, osmotic equilibrium depends on the fact that the sum of the ionic concentrations must be the same on both sides[2], *i.e.* $2x = 2y + z$, and from the nature of the case these two conditions are mutually incompatible. It can easily be shown that there will always be an excess concentration—and therefore a higher osmotic pressure—inside over that outside and therefore water will pass from the outer to the inner solution in order to equalise the concentration difference. This, however, will disturb the Donnan equilibrium

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[1] *Zeit. f. Elektrochem.* **17** (1911), p. 572.

[2] The concentration of undissociated acid will be the same on both sides and therefore does not affect the equilibrium. Also the osmotic pressure inside is really equal to $2y + z + a$ in which a is the molar concentration of colloid molecules and ions; a, however, is small and in a simple statement of the problem may be neglected. In the case of a protein a is proportional to the (very small) osmotic pressure of the pure protein, of the same molar concentration, at the isoelectric point. Cf. Loeb's book (*vide infra*) and *Science*, **56** (1923), pp. 731–741.

and more electrolyte will diffuse from the outer to the inner solution to readjust matters and this would further disturb the osmotic equilibrium. If the collodion bag is a closed one (or if it is connected only with a manometer) this diffusion of water and electrolyte inwards will continue until the tension on the walls of the bag (or the head of liquid in the manometer) is sufficient to just balance the excess of osmotic pressure inside over that outside. It is along these lines that the imbibition of water by gelatin and consequent swelling has been explained by Procter and his co-workers[1]. According to these workers the force which causes the entrance of water into a gelatin gel and thus determines the swelling is the osmotic pressure of the excess of crystalloidal ions inside over that outside the gel, this excess being due to the Donnan equilibrium. The opposing force which limits the swelling is the force of cohesion of the colloidal particles or gel. At equilibrium this opposing force of cohesion $(= e)$ must equal the osmotic pressure of the excess of crystalloidal ions inside over that outside the gel $= 2y + z - 2x$. The values of x, y and z can be determined experimentally and can also be calculated by means of the theoretical equations deduced by Donnan, Procter and Procter and Wilson. The agreement between calculated and observed values is satisfactory considering the difficulty of experimental work of this nature. A gelatin gel swelling under such conditions appears to behave as a perfectly elastic solid and obeys Hooke's Law, i.e. if $V =$ the increase in volume in c.c. of 1 mgm. equivalent weight of gelatin, $C =$ constant corresponding to the modulus of elasticity, and e, as above $=$ the osmotic force producing the swelling, then $e = CV$.

This relationship too has been shown to be in satisfactory agreement with the facts[2]. Now if the swelling of gelatin (or any other gel) is a linear function of the force producing the swelling it should follow that the diminution in volume of such a swollen gel under compression, as indicated by the amount of water retained, should be a linear function of the compressing force. Perhaps the simplest method of compressing a gel is by centrifuging as the compressing force can be easily varied by altering the speed of working. If the relationship holds then the water content after centrifuging should vary inversely as the centrifugal force employed. This is perhaps best expressed by the graphical method suggested by E. Buckingham[3] of plotting water content after centrifuging

[1] H. R. Procter, *J.C.S.* **105** (1914), p. 313; H. R. Procter and J. A. Wilson, *Ibid.* **109** (1916), p. 307; J. A. Wilson and W. H. Wilson, *Journ. Amer. Chem. Soc.* **40** (1918), p. 886.

[2] For a detailed treatment of this subject see J. Loeb, "The colloidal Behaviour of Proteins" (McGraw-Hill Book Co.), 1922.

[3] See Briggs and McLane, *loc. cit.*

for a given time against the reciprocal of the centrifugal force: the curve
obtained should be a straight line inclined to the horizontal axis. This
has not been done for gelatin and but few data seem to exist. Recently
Coward and Spencer[1] have measured the water retained by cotton
(which is known to swell in water) after centrifuging. Their results are
given in Table II, columns 1 and 3; in column 2 are given the reciprocals

Table II. *Water retained by cotton after centrifuging.*

Centrifugal force $\div g$*	Reciprocal of centrifugal force × 100,000	Water retained by cotton percentage of dry weight
2770	36·1	53
2460	40·65	55
2230	44·8	55
2170	46·1	57
2010	49·75	58
1400	71·4	65
1300	76·9	66

* g = acceleration due to gravity = 981 dynes; *i.e.* centrifugal force is expressed as a
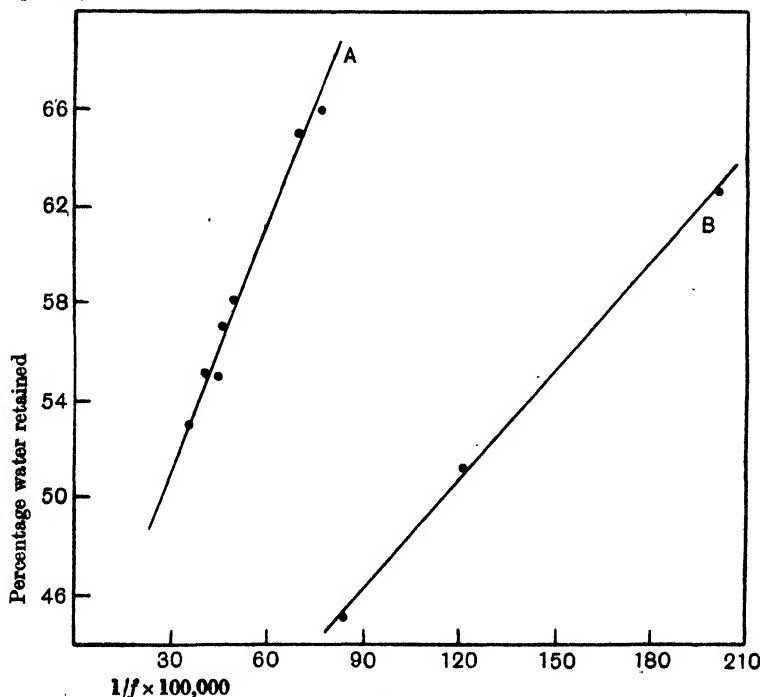multiple of g.

Fig. 1.

of the centrifugal forces × 100,000, and these are plotted against the
water content in Fig. 1, *A*. With a wool fabric the present writer obtained

[1] *Journ. Text. Inst.* (Trans.), **14** (1923), p. 28.

the results given in Table III and plotted in Fig. 1, *B*, from which it will be seen that in this case also the moisture content, *i.e.* the degree of swelling, is a linear function of the centrifugal force applied.

Table III. *Water retained by a wool fabric after centrifuging for 15 minutes.*

Centrifugal force $\div g$	Reciprocal of centrifugal force × 100,000	Water retained by wool percentage of dry weight	
450	202·0	61·24 ⎫ 62·75 ⎭	62·61
825	121·2	51·90 ⎫ 50·50 ⎭	51·20
1200	83·3	44·125 ⎫ 45·75 ⎭	44·94

Briggs and McLane[1] observed, but were unable to explain, a similar linear relationship in a number of soils they examined. Some of their data are collected in Table IV and plotted in Fig. 2. The curves shown

Table IV. *Moisture contents of soils after centrifuging at various speeds for 30 minutes.* (Adapted from Briggs and McLane.)

Speed in R.P.M.	2700	3000	3200	4100	4200	4300	5000	5500
Centrifugal force $\div g$	874	1050	1195	1961	2058	2157	2917	3529
$1/f \times 100{,}000$	114·4	95·2	83·7	51·0	48·6	46·4	34·3	28·3
Water retained by soil (percentage of dry weight)								
A. New Mexico Dune Sand	3·00	2·80	2·95	2·80	2·60	—	—	2·65
B. Sassafras loam, good	18·60	16·95	15·25	15·00	14·05	—	—	12·55
C. Leonardtown loam, good	18·05	16·50	15·20	13·60	14·20	—	—	12·10
D. „ loam, poor	12·00	10·30	9·65	7·75	7·40	—	—	6·3
E. Hagerstown stony loam	—	—	—	—	—	16·9	15·0	14·6
F. „ sandy loam	—	—	—	—	—	12·2	11·0	9·9
G. „ loam	—	—	—	—	—	18·3	17·5	16·5
H. „ silt loam	—	—	—	—	—	17·9	16·5	15·8
J. „ clay loam	—	—	—	—	—	26·0	25·5	24·8

are linear; all, except the one for dune sand, are sharply inclined to the horizontal axis and are roughly parallel. The equation for these curves is $m = m_0 + a/f$ in which m = moisture equivalent, m_0 = intercept cut off from the vertical axis by extrapolating to $1/f = 0$, f = the centrifugal force, and a = the slope of the curve. The average value of a for the curves given (excluding dune sand) is about 6100. Since an infinite centrifugal force should theoretically remove all water from a soil, all the curves on extrapolation should pass through the origin (the curve obtained on plotting e against V in the case of gelatin does so); since they do not Briggs and McLane inferred that the linear relationship must break down at large values of f.

[1] *Loc. cit.*

Really the distance of each curve above the horizontal axis depends upon the permeability of the soil, the value of the intercept at $1/f = 0$ being probably a rough measure of the deviation of $\dfrac{P_a\eta_a}{P_w\eta_w}$ from unity; the heavier the soil the greater is the intercept and the greater also is $\dfrac{P_a\eta_a}{P_w\eta_w}$. This is what one would expect; a small permeability would cause a large retention of capillary water as discussed above and hence
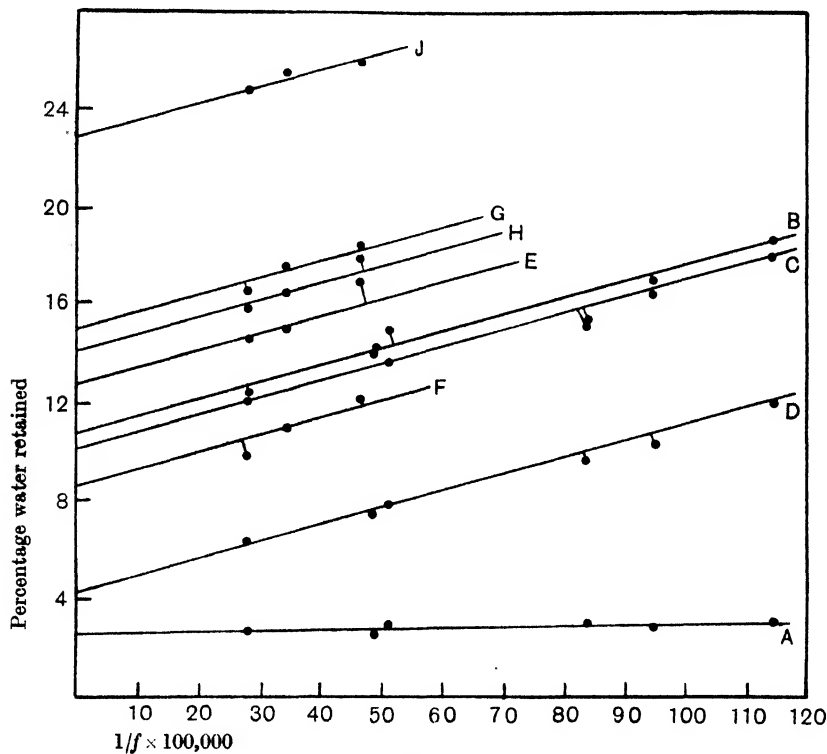
Fig. 2.

a large value for m_0, but one would not expect it to have much effect on the slope of the curve. From this point of view the slope of the curve may afford a measure of the *intrinsic swelling capacity* of the soil colloids especially if m and m_0 are expressed in percentage volumes instead of weights. Thus converting to a volume basis and dividing (for convenience) by 1000, the values of a for soil, wool and cotton, work out at 16, 16 and 50 respectively, *i.e.* wool and soil colloids swell to about

the same extent in water while cotton swells about three times as much[1]. The approximate constancy of slope, and hence presumably of swelling, with different soils is further supported by the work of W. O. Robinson[2] who has shown that the colloidal matter extracted from 34 soils of widely different origin and nature will absorb approximately the same weight of water, viz. from 0·240 to 0·348 gm. of water per gm. of dry colloid. With a material containing no colloidal matter and in which therefore there is no swelling in water, e.g. sand, a very slight slope only would be expected, viz. that due to the small though real effect of varying centrifugal force on the capillary moisture: this is indicated by the almost negligible slope of the dune sand curve (A in Fig. 2).

Further information might be obtained by using xylene (a liquid in which cotton, wool, soils, etc. do not swell) in place of water. Coward and Spencer[3], using a centrifugal force of 2230g found that 15 per cent. by weight = 26·1 per cent. by volume of xylene was retained by cotton as against 55 per cent. by weight = 82·5 per cent. by volume of water. The present writer has attempted to obtain a "xylol equivalent" —centrifugal force curve for the milled wool fabric previously dealt with but with little success, the variations between duplicate values being too great for any conclusions to be drawn. Thus, for a centrifugal force of 1200g the values for the xylol retained were 6·41, 5·46, 8·37, 7·97, 6·91 and 5·16, while for $f = 450g$ the amounts retained were 7·44, 6·54, 11·14, 8·16, 12·19 and 10·93 per cent.

Such large variations may be due partly to the difficulty of completely excluding moisture even from a xylol-saturated wool, but mainly to the difficulty of getting the wool hairs uniformly impregnated with xylol. Such difficulty is well known to biologists and, in the preparation of hairs for mounting with a view to section cutting, is overcome by soaking the hairs, sometimes under reduced pressure, first in alcohol and then in a series of alcohol-xylol mixtures of increasing xylol content, ending up with pure xylol. It is suggestive that the last four (high) values given above were obtained when the wool had been soaked in xylol under 2–3 cm. pressure. That much less xylol is retained than water is evident however even from these rough experiments and that

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[1] That cotton should swell apparently so much more than wool is possibly due to the fact that Coward and Spencer presumably used loose cotton in their experiments while the writer used pieces of milled (*i.e.* felted) wool fabric. In the latter case the strength and close packing of the milled fabric might be expected seriously to restrict the swelling of the individual wool hairs. If loose wool had been used the slope of the curve might have been much greater.

[2] *Journ. Phys. Chem.* **26** (1922), p. 647.   [3] *Loc. cit.*

this is the case with soils also is seen from a comparison of the moisture equivalents and the "xylol equivalents" of various soils given in Table V which were kindly determined for the writer by Dr A. F. Joseph.

Table V. *Moisture equivalents and xylol equivalents of various materials.*

Material	Moisture equivalent (ME)		Xylol equivalent (XE)		ME−XE Volume basis	Max. imbibitional water =1.38 ME	Percentage of total imbibitional water retained after centrifuging
	Dry wt. basis	Volume basis*	Dry wt. basis	Volume basis†			
Kaolin	41·2 40·8 } 41·0	106·6	33·0	99·4	7·2	147·1	4·9
Ball clay	47·2 45·5 } 46·3	120·4	20·2	60·9	59·5	166·2	35·8
Silty soil	23·5 22·6 } 23·0	59·8	8·2	24·7	35·1	82·5	42·5
Clay subsoil	51·6 47·8 } 49·7	129·2	13·8	41·6	87·6	178·3	49·1

* Calculation on basis of real specific gravity of soil = 2·60.

† Calculation on basis of real specific gravity of soil = 2·60 and specific gravity of xylol = 0·863.

In these determinations the moisture equivalents in duplicate were first determined by the standard method, the final dry weights being obtained by heating for 24 hours in an oven at 100° C. The moisture equivalents were then re-determined, again in duplicate, on these same oven-dried samples to see whether oven-drying had any appreciable effect on the determinations. These are the values given in Table V. They are slightly lower than those obtained with the original samples and are used here because the xylol equivalents can be determined only on the oven-dried samples and it is necessary to eliminate any effect of oven-drying on the moisture equivalent before one can legitimately compare the two sets of values, *i.e.* moisture equivalents and xylol equivalents. Any differences between these values, calculated on a volume basis, are probably a measure of the actual amounts of water imbibed by the colloid as distinct from the interstitial liquid which should not be very different in the two cases. It will be noticed that with kaolin which is notoriously feeble in, if not actually devoid of, colloid properties the moisture equivalent and xylol equivalent on a volume basis are almost identical. This seems to indicate that water and xylol are held by kaolin interstitially and that little, if any, actual imbibition takes place. The imbibition, and consequent swelling, is considerable in the other three cases and is much greater with the clay subsoil than with ball clay.

These results are interesting from another point of view. Wilsdon[1] inferred from experiments on the absorption of water by soil from sucrose solutions of different concentrations that the amount of water absorbed by 100 gm. of his particular soil was 11·5 gm. while the hygroscopic coefficient of the same sample, *i.e.* the amount of water absorbed per 100 gm. soil when in equilibrium with a saturated atmosphere, was 2·43 gm. The ratio between these two values is 4·73 which is in good agreement with the first constant of the Briggs-Shantz equation,

$$M = 4\cdot3H + 21,$$

in which M = maximum water retaining capacity and H = the hygroscopic coefficient of the soil. Wilsdon suggests that the term 21 in this equation represents the amount of "free" water in the soil, *i.e.* capillary or interstitial water[2], while the term $4\cdot3H$, or from Wilsdon's experimental results $4\cdot73H$, represents the total amount of water held by the soil colloids. Of the latter water Wilsdon distinguishes two kinds, one, the real hygroscopic moisture ($= H$ in quantity), is held by "adsorption" or in some other intimate manner by the colloids and is called "gel" water by Wilsdon, and the other ($= 3\cdot73H$) he describes as "vesicular water retained in the reticulated structure of the colloid." The value 4·73 he calls the "vesicular coefficient." From the foregoing considerations Wilsdon's vesicular water is the same as that called imbibitional water by the writer. Now according to other work of Briggs and Shantz we get $ME = 2\cdot7H$ in which ME = the moisture equivalent. Since $H = \dfrac{1}{3\cdot73} I$ in which I = the imbibitional water (*i.e.* Wilsdon's vesicular water) we have

$$ME = \frac{2\cdot7}{3\cdot73} I \text{ or } I = \frac{3\cdot73}{2\cdot7} ME = 1\cdot38 \, ME.$$

By multiplying the values of ME in column 3 of Table V by 1·38 we get the values given in column 7 for the imbibitional water held by the soil colloids when saturated. These values *minus* those given in column 6 represent the imbibitional water lost through centrifuging and, assuming no specificity on the part of the colloids, that is, assuming that the intrinsic swelling capacity of all soil colloids to be the same, the ratios of imbibitional water retained after centrifuging (column 6) to the total imbibitional water (column 7) should be the same in all cases

[1] *Mem. Ind. Dept. Agric.* (Chem. Series), **6**, No. 3, March 1921, p. 155.

[2] Wilsdon attempted a mathematical derivation of the term 21 but his reasoning has been shown to be fallacious by B. A. Keen (this *Journal*, **14** (1924), p. 171). The fallacy does not appear to affect that portion of Wilsdon's argument used in the present discussion.

and are given in column 8. The actual values are 4·9 for kaolin, 49·1 for the clay subsoil, 42·5 for the silty soil and 35·8 for the ball clay. The value calculated from Wilsdon's values should be 47 per cent. The agreement (excluding kaolin) is perhaps as good as could be expected in view of the diversity of the materials used. The "constants" appear to be constant only for closely related types of soil and may have widely different values for different types[1]. The relationship seems to break down altogether with kaolin in which the imbibition is almost negligible.

The significance of the vesicular coefficient in the Briggs-Shantz equation is of some little interest and importance, although at present we know nothing of its real nature. In the original equation it is merely an empirical constant based on a large number of experimental determinations of the values of M and H. Wilsdon points out that it expresses the ratio between the weight of water absorbed by 100 gm. of soil in contact with liquid water and the weight absorbed when the soil is in contact with a saturated atmosphere. This ratio, it is maintained by both Wilsdon and Alway, should theoretically be equal to unity. This is correct for a material like sand, but it is not necessarily correct for colloidal systems, because with gels there may be factors operating which cannot be brought within the scope of the classical theory. The phenomenon, too, is not confined to soils. As long ago as 1903 von Schroeder[2] found that a gelatin gel when allowed to swell *in water* would imbibe over 1000 per cent. of water; the same (unswollen) sample exposed at the same temperature to saturated water vapour would not absorb more than about 400 per cent. Moreover, the swollen gel when transferred from water to saturated vapour would lose water and von Schroeder to account for this phenomenon concluded that the vapour pressure of water in a swollen gelatin gel must be higher than that of pure water—a conclusion that will be shared by few. The phenomenon was attributed entirely to experimental error by Wolff and Buchner[3] but the problem is too similar to the corresponding soil one to be dismissed so easily. The point has also been investigated by Katz[4], by von Gericke[5], by Bancroft[6] and by Shull[7], and the phenomenon—known as von Schroeder's paradox—appears to be well established.

E. W. Washburn[8], again, found that moistened clay can be dried to

[1] Alway and Clark, *Journ. Agric. Res.* **7** (1916), p. 345; F. Hardy, this *Journal*, **13** (1923), pp. 243, 340.

[2] *Zeit. phys. Chem.* **45** (1903), p. 75.   [3] *Ibid.* **89** (1915), p. 271.

[4] *Koll. Beihefte.* **9** (1917–18), p. 1.   [5] *Koll. Zeit.* **17** (1915), p. 78.

[6] *Journ. Phys. Chem.* **16** (1912), p. 396.   [7] *Amer. Journ. Bot.* **7** (1920), p. 318.

[8] *Journ. Amer. Cer. Soc.* **1** (1918), p. 25.

a considerable degree if suspended in a closed vessel over water, but, as pointed out by Bancroft[1], his explanation of this as a gravity effect is demonstrably unsound.

These similar phenomena in soils, ceramic clay and gelatin are difficult of explanation on any classical physical-chemical basis, and do not appear to be a consequence of the Donnan equilibrium. The swelling of gelatin in very dilute acids and alkalies, as pointed out above, can be satisfactorily explained on the basis of a Donnan equilibrium, but it is obvious that the presence of an outside solution of lower concentration than, but continuous with, the inner solution is a necessary condition for the attainment of such an equilibrium. Once attained, however, the mere removal of the outer solution should not affect the swelling unless some new factor is brought into play.

It is not suggested that the imbibition of water by colloid gels is due in all cases to the existence of a Donnan equilibrium; in cotton at any rate it is difficult at present to accept such an explanation although it cannot be definitely rejected until we understand more fully the part played by the ash content in the colloidal behaviour of such (carbohydrate) gels[2]. In the case of wool (a protein system) and clay (and soil colloids generally) such an explanation is quite a possible one and is worthy of thorough and systematic investigation. O. Arrhenius[3] has recently suggested that clay is an ampholyte although his experiments are too scanty and limited to be more than merely suggestive. There is no doubt that clay is a very reactive substance and much of the characteristic behaviour of soil can be explained as well on a purely chemical basis as from the more popular colloid or adsorption standpoint[4]. It is hoped to investigate further this point of view with both wool and soil systems.

It is perhaps worthy of note that the forces that cause imbibition whether due directly to Donnan equilibrium or not must also be operative in the movement of water within the soil mass. The movement of soil water has been the subject of much attention on the part of numerous investigators, notably Briggs, Buckingham, King, Schlichter, Gardner and others, but these workers have dealt with the problem mainly from the capillary point of view, regarding the soil as a collection of solid particles between which the movement of water is dependent entirely

[1] *Applied Colloid Chem.* (McGraw-Hill Book Co., London, 1921), p. 76.

[2] In this connection see "The Swelling of Agar Agar," F. Fairbrother and H. Mastin, *J.C.S.* **123** (1923), p. 1412.

[3] *Journ. Amer. Chem. Soc.* **44** (1922), p. 521.

[4] E. A. Fisher, *Trans. Far. Soc.* **17** (1922), p. 305.

on the action of gravity, friction, hydrostatic pressure, surface tension and so on. This point of view has been further developed mathematically by Gardner[1] in a very general treatment with which the point of view indicated here is not inconsistent. Thus he points out that movement of soil water depends on the existence of a potential gradient and the potential function Φ involved in his equations is made up of the sum of three independent terms: π = energy due to hydrostatic pressure; ψ = energy due to capillary pressure; and ϕ = energy due to gravity. Since the negative gradient of this potential becomes a force per unit mass, ρ = moisture density, *i.e.* the aggregate amount of water present in unit volume of soil, must come in as a factor. He goes on to say

"the mean velocity of any given portion of liquid· through the soil is proportional to the pressure gradient, or in equivalent language, to the product of the amount of moisture in unit volume at the point in question and the rate of change of the potential energy per unit mass characteristic of such point as we move from point to point in the given region. …While the velocity of the element of liquid may ultimately depend upon a large number of factors such as the surface tension of the liquid, the coefficient of viscosity of the liquid, the porosity of the soil, the size and shape of the individual grains, the temperature, the barometric pressure, and so on, yet the effect of each will be made manifest through one or other of the three quantities ρ, Φ $[= \pi + \psi + \phi]$ and K" [= the transmission constant of Schlichter].

It is impossible to measure capillary pressure directly and an attempt was made by Gardner to evaluate it in terms of ρ, *i.e.* the moisture content, and in another paper[2] he showed that the work of Briggs and McLane discussed above indicates that the capillary potential, ψ, is a linear function of the reciprocal of the moisture content, ρ, over a considerable range, *i.e.*

$$\psi = \frac{c}{\rho} + b.$$

What Gardner calls the capillary potential may, therefore, include factors not necessarily capillary in character such, for example, as the cohesion of the clay particles, or of the colloidal coating of the soil grains, which would result in a force of compression on the imbibitional water equal (on the hypothesis discussed above) to the difference in osmotic pressure inside and outside the colloid. Thus, assume a portion of moist, not wet, soil in equilibrium. If the moisture content is diminished, say, at the surface, by evaporation this loss would fall largely on the im-

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[1] *Soil Science,* **11** (1921), p. 215.

[2] *Ibid.* **10** (1920), p. 357.

bibitional water as shown by the writer[1]. This would result in a diminution in pressure on the imbibitional water of the surface gel owing to lessened distention and (imbibitional) water would in consequence pass from the interior to the surface of the soil, until equilibrium was again attained. This process would be much slower than the corresponding capillary movement but would be expected to be faster with the lighter, more porous soils, because in the latter case the lost imbibitional water might be replaced by capillary water which in turn would be rapidly replaced by more capillary water from below which in turn would be replaced by capillary *and* imbibitional water from the interior of the mass. With heavy soils of low porosity capillary movement may be almost entirely replaced by what may be called "imbibitional" movement[2]; such considerations may in part explain the rapid initial rise in coarse textured sandy soils and the very much slower rise but higher final values in fine textured clay soils such as those given by Loughridge for Californian soils[3].

SUMMARY.

It has been shown in earlier papers that the water present in soils is held in two ways: as capillary or interstitial water present as water wedges between the soil grains and as imbibitional or "gel" water which is associated in some way other than interstitially with the clay particles and with the colloidal coating of the soil grains. Imbibitional water is the cause of the swelling in water of soil and other colloidal (gel) systems such as cotton, wool, gelatin.

The nature of the imbibitional process is discussed and it is suggested that with clay, wool and soil imbibition may be due to the attainment of a Donnan equilibrium as is the case with gelatin. Experiments are quoted and discussed in support of this suggestion; and in particular it is pointed out that such swollen colloids appear to behave as perfectly elastic solids when under compression. This is not the case with sand and water nor with soil, wool or cotton in xylol, *i.e.* when no swelling occurs.

Imbibition may be a factor in the movement of soil water, in addition to capillarity, and it is shown that such a factor is quite consistent with the general mathematical treatment of the movement of soil water as developed by Gardner and others.

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[1] *Roy. Soc. Proc.* **103** A (1923), p. 664.

[2] That the presence of a colloid phase may, and probably does, affect the rate of movement of soil water has been recognised by Wilsdon (*loc. cit.*) but his point of view is somewhat different from the writer's.

[3] Quoted by B. A. Keen, this *Journal*, **9** (1919), p. 396.

[It is realised that the point of view outlined above rests, as far as soil is concerned, on a very slender experimental basis, but it appears to be of sufficient interest to merit further investigation. The writer, however, has neither facilities nor opportunity for developing the matter further and this paper is published in its present form in the hope that it may prove of some interest to other workers more directly connected with soil research.]

AN ELECTRICAL METHOD FOR THE REDUCTION OF DRAUGHT IN PLOUGHING.

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(With Six Text-figures.)

AN appreciable fraction of the total work done in ploughing arises from frictional forces between the mouldboard and the soil. Although no exact data are available, this amount has been estimated as one-third. The problem of keeping this friction as small as possible has led the manufacturers to produce mouldboards which will "scour" well by selecting special classes of steel and methods of tempering. In the following paper a simple electrical method is proposed for the reduction of friction on moist substances and an account is given of a preliminary investigation into its possible application to ploughing. The method proposed depends upon the phenomenon of electroendosmose which is exhibited by moist soil. In virtue of the negative charge of the soil colloids, water will move through moist soil towards the negative electrode under the action of an electric current. It is suggested that, if a current be passed through the soil having the mouldboard of a plough as negative electrode, then the film of water formed at the soil-metal surface should act as a lubricant and reduce the ploughing draught.

Striking reductions of friction were obtained by the use of such a current in laboratory experiments. A slider consisting of a weighted metal spatula was drawn over the surface of a slab of moist soil in a metal tray by means of a thread passing over a pulley to a scale pan. Weights were added to this scale pan until the tension in the thread was sufficient to keep the slider in steady motion. The weight thus determined was taken as a measure of the friction between the spatula and the soil. A current from a 4-volt accumulator could be passed through the soil, by wires attached to the slider and the tray, a reversing switch being included in the circuit. Some of the results obtained in this way are illustrated in Fig. 1. The first measurements with no current gave a value of just under 600 gms. At a time corresponding to the point

marked *A*, the current was applied with the slider positive, thus causing a drying of the soil in contact with the spatula and the pull required rose rapidly to 1500 gm. The current was then switched off for a short period and the friction fell (*BC*). The fact that the value did not fall to its initial magnitude is ascribed to the slowness of the return of water to the area
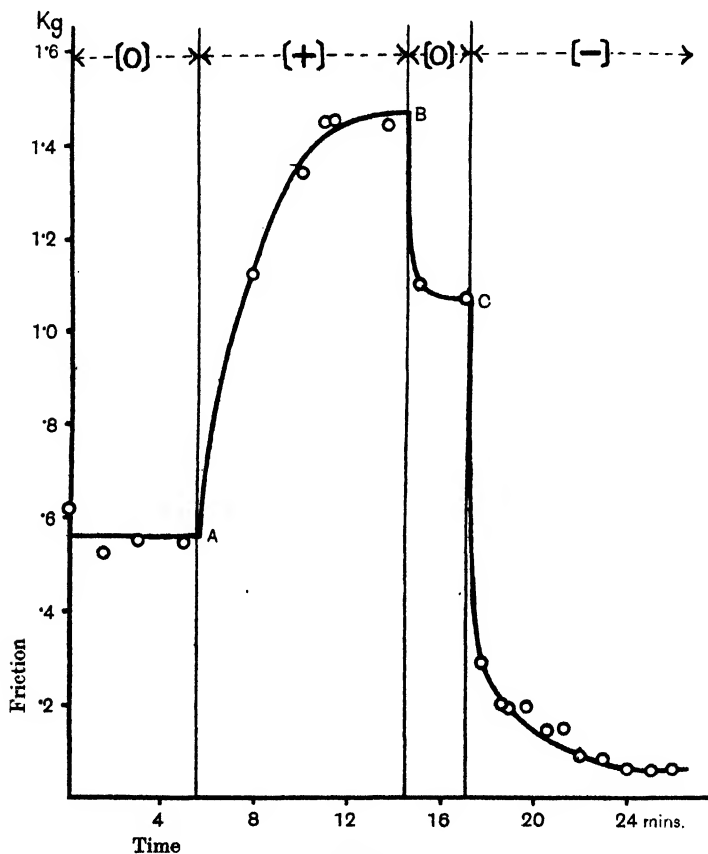
Fig. 1.

which had been dried by the current. At *C* the current was applied with the slider negative and the friction fell very rapidly to half its normal value, while after some minutes it had reached a value as low as 50 gm. With loads below the normal it was possible to stop or start the motion of the spatula by switching the current off or on. By applying the current for some minutes the soil could be made almost dry or completely flooded with water according to the direction of the current.

The rapid response to the application of the current suggested the

measurement of the time necessary for the current to cause a definite reduction in friction. The results of such an experiment are represented in Fig. 2, which shows the weights necessary to move the slider, starting from a position of rest. A series of measurements without current gave values of about 300 gm. The weight in the pan was reduced to 100 gm. and the current applied, with the slider negative, for an interval just sufficient to cause the slider to move. This interval was found to be six seconds; in this time the current had reduced the friction to one-third.
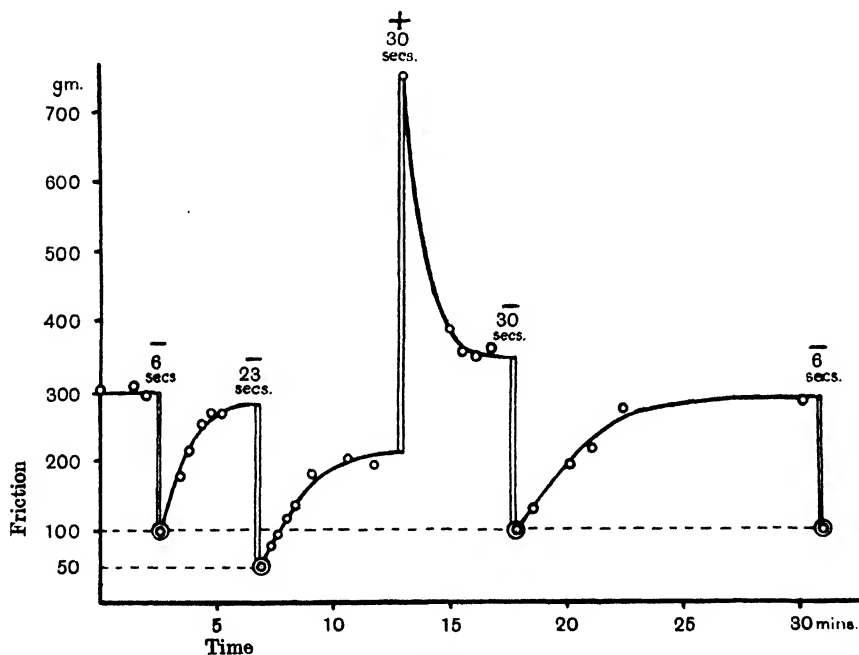
Fig. 2.

Measurements were continued without the current, the friction remaining low and only slowly increasing towards its normal value. When this had been approximately regained, the experiment was repeated with 50 gm. in the scale pan, when the current was required for 23 seconds. In the next test the current was applied for 30 seconds, with the slider positive, and the friction rose to 750 gm. After allowing time for the slow return of the friction to its normal value, the first test was repeated twice, showing reductions of the friction to 100 gm. by the passage of the current, with slider negative, for intervals of 30 seconds and 6 seconds respectively.

Since it was almost impossible to maintain a steady motion of the slider over the soil with the apparatus already described, an improved form, shown in Fig. 3, was devised for more extensive measurements. In this case the slider was kept stationary whilst the soil slab was moved by having it mounted in a light metal carriage on rails. The slider consisted of a loaded triangular stage supported on three sliding castors of the type known commercially as "Domes of Silence." A thread
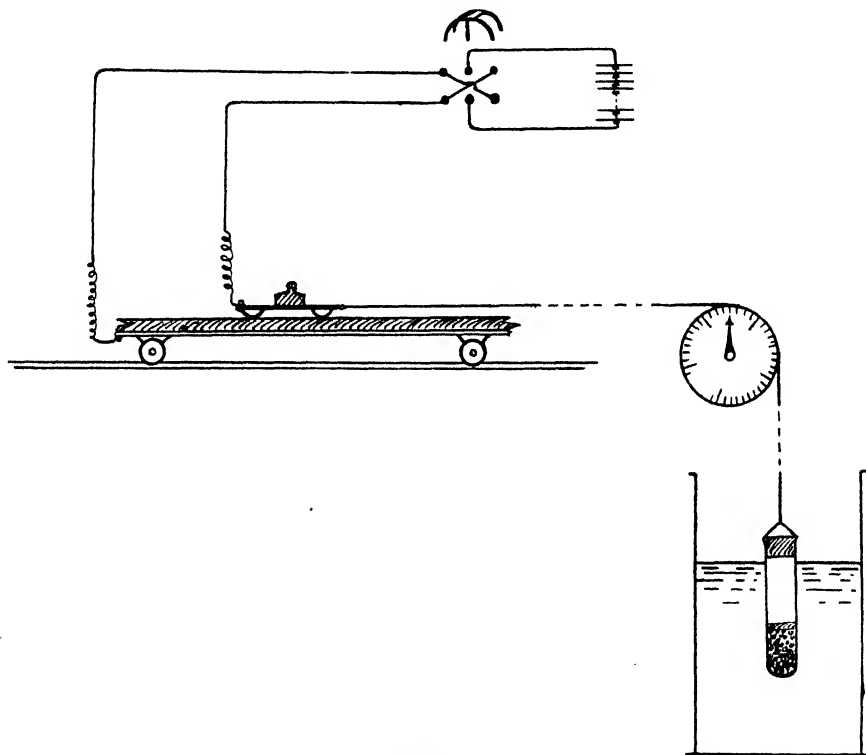
Fig. 3.

attached to the slider passed over a pulley to a float in water. Referring to the figure it will be seen that, if the soil carriage is moved to the left at a uniform rate, the slider will be dragged with it until the frictional force is just balanced by the effective weight of the float. Since this is proportional to the length of the float lifted out of the water, and, therefore, to the movement of the thread, the tension applied to the slider can be read off from a suitable scale marked on the circumference of the pulley wheel.

In order to reduce errors due to a progressive smoothing of the soil surface by the repeated passage of the slides, readings were taken alternately with and without the current, the slider being negative in all cases. From the mean of four such pairs of measurements the percentage reduction in friction due to the current was determined for a series of voltages and at different water contents of the soil. Fig. 4 shows the results of such a series on a Rothamsted heavy loam; a heavy clay soil behaved similarly. The extent of the reduction of friction is intimately connected with the water content of the soil. Below 14 per cent. moisture
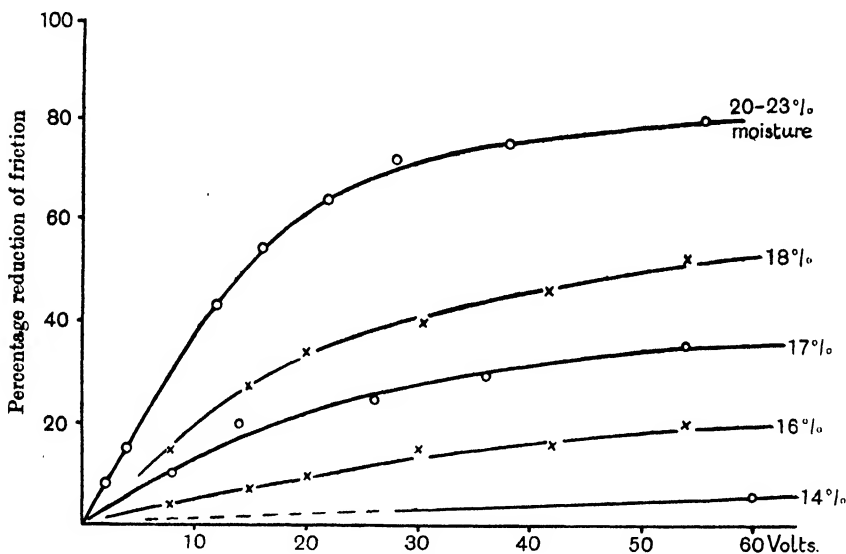
Fig. 4.

the effect is almost negligible, but it increases rapidly as the soil becomes wetter, giving a maximum reduction of 80 per cent. by the application of 50 volts to a soil with 20 per cent. moisture. At 23 per cent. moisture the results were almost identical with those at 20 per cent., so that at these water contents the movement of the soil water is so free that a further addition causes little change in its movements in response to an applied potential. The "lubricating action" of the water film is obviously closely connected with the current density and with the duration of contact between soil and metal. The resistance of the soil block was measured with an alternating current during the actual movement of the slider over the soil and gave for water contents of 23 per cent., 20 per cent., 17 per cent. and 14 per cent., resistances of 520, 530, 1000 and 2500 ohms respectively. The area of contact between the slider and soil was

about 0·5 sq. cm. so that with an applied potential of 50 volts the current densities were 0·2, 0·2, 0·1 and 0·04 amps. per sq. cm. respectively. In all the experiments represented in Fig. 4, the relative motion of the slider and soil was 4 feet per min. (2 cm. per sec.), and with a range of speeds from 1 to 16 feet per min., the percentage reduction in friction was found to be approximately inversely proportional to the speed, or directly proportional to the duration of the contact at any point on the soil surface.

Under the normal conditions of ploughing, the water content of the soil will usually be of the same order as that showing marked effects in the laboratory, but large changes will occur in the magnitudes of the other factors involved. A comparison between the dimensions and speeds of the average plough mouldboard and the slider of the experiments just described shows that the area of contact is increased 5000 times, but the duration of contact of any point in the soil surface with the metal is increased ten times. Under field conditions the current must therefore be increased 500 times to give results similar to those obtained in the laboratory. Thus, a 40 per cent. reduction, shown in Fig. 4 for soil with 20 per cent. moisture and a current of 0·02 amp. (10 volts), would require 10 amps. applied uniformly over the mouldboard. If the current be concentrated in the region of the ploughshare, advantage can be taken of the appreciable time lag which has been shown to occur in the return of a soil surface to its normal state after the application of the current. The lubricating effect will be greatest at the point of greatest friction, and the water film may be expected to remain operative as the soil passes over the rest of the mouldboard, and so compensate for a low average current density. This object would be attained by using the coulter as the positive electrode. The increased friction which inevitably occurs at the positive electrode, would be small with this arrangement, since the surface area of a knife coulter is small, whilst the total friction with a revolving coulter is a minimum. Simple modifications of an ordinary plough therefore rendered it possible to test whether the effect occurs under field conditions.

The general arrangement of the field tests was the same in all cases and involved only the insulation of the coulter from the plough-frame by packing with fibre. Current was obtained either from a stationary generating set and a trailing cable or from a dynamo carried by the tractor. The draught of the plough was measured by a Watson Draw-Bar Dynamometer. This instrument gives a continuous record on a chart of (1) draw-bar pull, (2) distance travelled, and (3) depth of ploughing. Normally it has an electrical timing device, but, for these

experiments, this was replaced by a hand-switch so that the exact point at which the current was applied or disconnected could be recorded on the chart. The field chosen was divided up along the furrows into plots of equal length (either 33 feet or 66 feet). Two white stakes suitably separated were erected on each dividing line and a similar stake was fixed to the plough. An observer placed himself in alignment with the two stakes and was able to time accurately the moment at which the plough crossed the dividing line. The time taken in ploughing each plot was measured by the observer by means of two stop-watches arranged so that the action that stopped one started the other. At the given signal a second observer operated the switches for the current supply and for marking the dynamometer chart. Ammeter readings were taken over each plot. The method of experiment was to drive the tractor as steadily as possible along the whole furrow and have the current alternately on and off during successive plots.

The electrified and control plots were compared on the basis of (i) draw-bar pull (D.B.P.) and (ii) a quantity which we have called "power." The latter term was introduced, as some uncertainty was felt in relying on the D.B.P. values alone, since these omit the time-factor. It seems probable that, with an engine working at constant output, any reduction in soil resistance would be reflected in increased speed as well as in reduced D.B.P.[1]. Hence the D.B.P. figures were multiplied by the time taken and the resulting values treated as power consumption. A facsimile of a portion of the dynamometer chart is given in Fig. 5, the electrified plots being shaded. It is obvious that, in any cultivation measurements of this type, changes in soil texture and other factors cause such large and erratic fluctuations in D.B.P. that special methods of treatment are necessary to eliminate errors. Some form of "micro-plot" method of comparison is at least as essential here as in field experiments on crops where small differences are looked for, together with some measure of their reliability. The method of experiment was arranged to give 16 separate plots along each furrow. It is difficult to ensure uniform behaviour of the tractor and plough over successive furrows and it was occasionally necessary to make some readjustment at the end of a furrow. Each furrow was therefore treated as a separate experiment and each plot compared with the mean of the two adjacent plots (which had, of course, the opposite treatment). In this way all the plots, with the exception of the end ones, were taken twice over.

[1] This matter will be discussed in detail at a later date in a communication from the Physical Department at Rothamsted.

The method of treating the results is shown in Table I which gives data for the chart in Fig. 5. The mean value of the draw-bar pull was determined for each plot by integration of the dynamometer chart with a Conradi Rolling-type Planimeter. From the percentage decrease or

Table I.

| | | Dynamometer chart readings | | | | | Comparisons | | | | | |
| | | | | | | | D.B.P. | | | Power | | |
Plot No.	Current	Area under curve	Length cm.	Mean D.B.P.	Time sec.	Power D.B.P. ×time	Control	Electri-fied	Percent-age (con-trol, 100)	Control	Electri-fied	Percent-age (con-trol, 100)
1	Off	644	1·63	395	21·2	838	—	—	—	—	—	—
2	On	657	1·57	418	19·4	812	416	418	100·3	813	812	99·9
3	Off	713	1·63	437	18·0	788	437	426	97·4	788	780	98·9
4	On	705	1·62	435	17·2	748	456	435	95·4	803	748	93·2
5	Off	743	1·56	476	17·2	819	476	446	93·7	819	786	95·9
6	On	741	1·62	457	18·0	824	479	457	95·5	824	824	100·0
7	Off	747	1·55	482	17·2	829	482	468	97·1	829	805	97·1
8	On	787	1·64	480	16·4	787	494	480	97·3	830	787	94·8
9	Off	775	1·53	506	16·4	831	506	465	92·0	831	758	91·2
10	On	726	1·61	451	16·2	730	483	451	93·4	798	730	91·4
11	Off	678	1·47	461	16·6	766	461	451	97·9	766	713	93·2
12	On	732	1·62	452	15·4	696	441	452	102·5	715	696	97·3
13	Off	642	1·49	431	15·4	664	431	462	107·2	664	726	109·4
14	On	743	1·57	473	16·0	757	491	473	96·3	754	757	100·3
15	Off	814	1·60	509	16·6	844	509	481	94·5	844	775	92·9
16	On	794	1·62	490	16·2	794	—	—	—	—	—	—

Mean 97·2   Mean 96·8
Gain 2·8 %   Gain 3·2 %
Probable error± 0·7   Probable error± 0·8
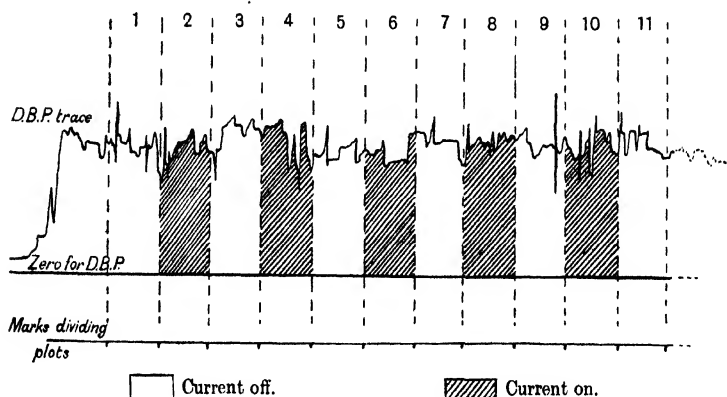
Fig. 5.

increase shown by each comparison, a mean value with its "probable error" was calculated for the furrow. The method of comparison is illustrated graphically in Fig. 6 for a series of four similar furrows. The shaded triangles show the decrease in draw-bar pull when the electrified

plot is compared with the two adjoining controls; an increase in D.B.P. is shown by an unshaded triangle. The preponderance of the shaded portions indicates that for these furrows there is evidence of a decreased D.B.P. due to the current.
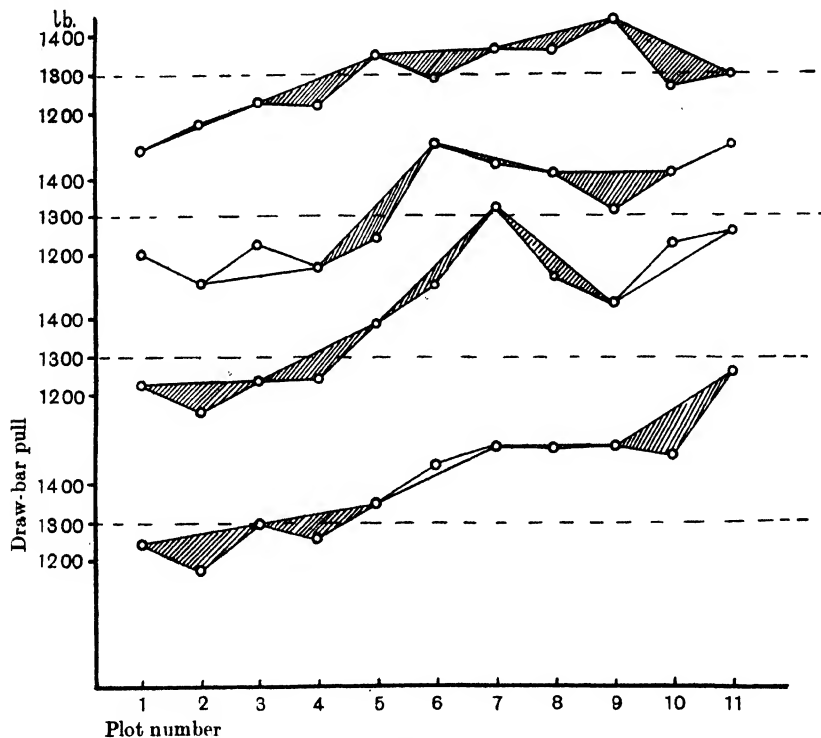
Fig. 6.

Table II.

		No. of plots	Percentage difference due to current	
Test			D.B.P.	Power
I. Rothamsted (Oct. 1922)		16	$-2\cdot9\pm0\cdot7$	$-6\cdot2\pm1\cdot1$
		16	$-2\cdot2\pm0\cdot7$	$-5\cdot0\pm0\cdot9$
II. Rothamsted (Jan. 1923)		16	$-2\cdot8\pm0\cdot7$	$-3\cdot2\pm0\cdot8$
		16	$-0\cdot6\pm0\cdot6$	$-1\cdot0\pm1\cdot1$
		16	$-3\cdot9\pm0\cdot8$	$-5\cdot0\pm1\cdot7$
		16	$-1\cdot5\pm0\cdot9$	$-5\cdot6+1\cdot6$
III. Lingfield	(28 Mar. 1923)	24	$-3\cdot9$	$-1\cdot6\pm2\cdot4$
		72	$-1\cdot8$	$+0\cdot3\pm1\cdot4$
IV. Lingfield	(26 Mar. 1923)	21	$-0\cdot9\pm1\cdot3$	$-5\cdot8\pm0\cdot9$
		18	$-3\cdot8\pm1\cdot3$	$-3\cdot9\pm1\cdot6$

The results of four series of experiments carried out on two different soils are summarised in Table II. In the first two groups the soil was

a heavy loam in Great Knott Field at Rothamsted. A single-furrow plough was connected by a trailing field telephone cable with a 110 volt D.C. 1½ kilowatt generating set. Some furrows have been omitted from the table as being unreliable, generally because of readjustments or stoppages occurring in the middle of a run: in every case the discarded furrow gave a very high "probable error." The water content of the soil in both cases was below that usually present during ploughing. The current varied between 2 and 4 amps., corresponding to an effective resistance of 25 to 50 ohms.

The second series of tests was made on a silty soil at Greater Felcourt Farm, Lingfield, Surrey, by the courtesy of Mr R. Borlase Matthews, who placed at our disposal the excellent electrical facilities with which he has equipped this farm. A three-furrow plough drawn by an Overland tractor was used. In Test III the current was led from a main overhead supply at 440 volts by a 9/16 cable. The field had carried mangolds and was too wet for good ploughing; it is probable that the water film already present masked the effect of that produced by the current. The mean current was about 4 amps. per plough giving an effective soil resistance of about 100 ohms. Test IV was of a different and more practicable type. A self-contained unit was formed by having a 110 volt dynamo mounted on and driven by the tractor. On the electrified plots the tractor engine had the extra load imposed by the dynamo. In spite of this it was noticed that the engine "eased up" over the electrified plots and the time records showed that the speed of ploughing was increased. The current in this test was 1–2 amps. per plough, giving again an effective resistance of rather less than 100 ohms. In both Lingfield tests, therefore, the soil resistance was considerably greater than at Rothamsted in spite of the much higher water content of the soil.

As an additional check, a few tests were made with the mouldboard as positive electrode, with the result that the draw-bar pull and power were increased. It is possible that a small effect arises from the electro-static attraction between the electrified surfaces, which would increase the draught independently of the direction of the current.

An examination of the collected results in Table II indicates fair grounds for claiming that the device has succeeded in reducing the work done in ploughing. The amount of the reduction obtained in these experiments is so far too small to have any great practical significance, since the gain would be outweighed by the cost of generating the electricity and delivering it to the plough. There is, however, considerable possibility of improvement in the method of applying the current; hitherto we have

made use only of the accidental facilities inherent in plough design. We hope to be able to carry the work further and investigate such questions as the effect of subsidiary electrodes placed closer to the mouldboard. It appears probable that the process would have more useful application on special types of soil, such as those which "scour" badly (*i.e.* tend to stick to the mouldboard), or for certain other cultivation processes such as mole-drainage and deep ploughing. Further, a possible development of cheap electricity supplies and the more extended use of electrical power for soil cultivation may render the method of practical value.

SUMMARY.

An electrical method is suggested for reducing the friction between a metal surface and soil, or other moist substances; and its possible application to soil cultivation is considered.

Large reductions in friction were obtained in laboratory tests with a metallic slider moving over moist soil.

Preliminary tests in the field demonstrated that the draught of a plough was reduced by applying a current between the coulter and the mouldboard. The magnitude of the reduction obtained with this arrangement was too small to have immediate practical value, but the possibility of increasing the effect is discussed.

ON THE MEASUREMENT OF HYDROGEN-ION CONCENTRATIONS IN SOIL BY MEANS OF THE QUINHYDRONE ELECTRODE.

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THE measurement of the hydrogen-ion concentration of soils often presents great difficulties. When ordinary hydrogen electrodes are used, constant potentials are, in many cases, only obtained after hydrogen has been passed for several hours, while in many instances hydrogen electrodes cannot be used at all. The other important method of determining "pH," the colorimetric method, can only be used in testing clear and almost colourless soil extracts, but not in testing soil mixtures.

When the use of the usual hydrogen electrode presents difficulties, this is doubtless often due to the presence of reducible substances, which act as depolarisers at the electrode, until they are gradually reduced by action of the hydrogen, whose effect is catalysed by the platinum-black of the electrode.

In 1920 I demonstrated that by means of the organic compound quinhydrone[1] it is possible to form an electrode which acts as a hydrogen electrode with exceedingly low hydrogen pressure. The quinhydrone is a combination of 1 molecule quinone $C_6H_4O_2$ with 1 molecule hydroquinone $C_6H_4O_2H_2$. In aqueous solution it is extremely dissociated into these components. Further, an equilibrium exists which may be expressed by the equation $C_6H_4O_2H_2 \rightleftharpoons C_6H_4O_2 + H_2$. The hydroquinone acts in the solution as a hydrogen source, whose effect corresponds to a "hydrogen pressure," which at 18° C. is $10^{-24 \cdot 4}$ atmospheres, at 25° C. $10^{-23 \cdot 6}$ atmospheres. These values are found by experiment; for an element formed of a platinum plate placed in a solution of quinhydrone in a dilute electrolyte and a hydrogen electrode placed in the same electrolyte (hydrogen pressure = 1 atmosphere) has a voltage of 0·7044 at 18° C. and 0·6990 at 25° C. This potential is independent of the electrolyte and of the concentration of quinhydrone.

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[1] København's Universitets Festskrift, 1920. *Annales de Chimie*, 9. serie, **15**, 109 (1921). Biilmann and Lund, *ib.* **18**, 321 (1921).

The Quinhydrone Electrode, as I have named this electrode, may be used in acid solutions and, in the case of soil-water mixtures, also in basic solutions up to pH ca. 8·5[1]. In other words, the quinhydrone electrode may be used in the entire field which has practical interest in soil investigations.

The preparation of the quinhydrone electrode is extremely simple and quick. Use ordinary electrode vessels of the shape used for calomel electrodes. Shake ca. 20 c.c. of the solution or mixture to be examined for a few seconds in a test-tube with a few centigrams of quinhydrone. Pour the mixture into the electrode vessel and place in it an electrode made of bright platinum. As soon as the solution in the electrode vessel has the desired temperature, the potential may be determined, for the quinhydrone at once gives the corresponding hydrogen pressure. If we measure an element formed of a quinhydrone electrode and a decinormal calomel electrode combined by means of a saturated solution of KCl, at 18° C. we have

$$pH = 6·35 - \frac{\pi}{0·0577},$$

in which π is the measured potential of the quinhydrone electrode in relation to the calomel electrode. From the expression given above for pH we see that this potential becomes zero when pH of the examined solution is 6·35. With a lower value of pH, π is positive; with a higher, negative.

The standard calomel electrode may be replaced with advantage by a standard quinhydrone electrode, described by Stig Veibel[2]. The element thus formed is represented by

Pt	Quinhydrone HCl 0·01 N KCl 0·09 N	KCl saturated	Quinhydrone soil + water	Pt

and the potential (π) is positive for all soil samples in practice. At 18° C. pH is given by

$$pH = 2·04 + \frac{\pi}{0·0577}.$$

It could not be taken for granted that the quinhydrone electrode was applicable to soil tests. Though the soil itself may possess considerable

[1] In earlier investigations I have only used the quinhydrone electrode in acid solutions, but during the present work it was found that the electrode may also be used in the weakly alkaline mixtures of soil and water. Recently Kolthoff (*Rec. Trav. Chim. Pays Bas*, **42**, 186, 1923) has used the electrode at pH 8. [2] *Journ. Chem. Soc.* **123**, 2203, 1923.

buffer effect, the soil extract has normally a very slight buffer effect. And as hydroquinone, one of the components of the quinhydrone, is acid, though very slightly so, it was conceivable that quinhydrone might change the pH of the soil extract so much that measurements made with quinhydrone electrodes would give false values. On the other hand the quinhydrone electrode has this great advantage, that its reducing effect is far less than that of the usual hydrogen electrode. As a matter of fact, with the quinhydrone electrode very constant potentials are obtained not only in 0·1 normal nitric acid, but also in a mixture of equal volumes of 0·1 normal nitric acid and 0·1 normal hydrochloric acid. Whilst the presence of nitrates will, as is known, prevent the application of the ordinary hydrogen electrode, this is not the case with the quinhydrone electrode. In making the soil tests the importance of this fact is evident.

Together with Mr Hakon Lund I have tried to measure hydrogen-ion concentrations in soil extracts and in mixtures of soil and water by means of the quinhydrone electrode.

We first investigated how well the potentials of the electrode were reproduced in soil extracts. The extracts were prepared from 100 gm. soil mixed with 100 gm. distilled water. The mixture was placed in a corked glass, shaken repeatedly and left to stand overnight. The resulting extracts was either used directly, or if colorimetric comparisons were to be made, after filtering.

Extracts of three different soil samples, S 1, S 2 and S 3 were used, and each sample was tested with three quinhydrone standard electrodes I, II and III. Table I gives the potential difference in volts between these electrodes and the standard quinhydrone electrode.

Table I.

	I	II	III
S 1	0·3220	0·3222	0·3226
S 2	0·3365	0·3340	0·3360
S 3	0·3355	0·3355	0·3340

The greatest difference between the potentials found for the same soil extract is here 0·0025 volt. Since a difference of 0·1 in the value for pH corresponds to 0·00577 volt, it must be admitted, that the potentials are reproduced with an agreement which entirely satisfies the requirements of soil investigations.

As it is often quite difficult to filter considerable quantities of soil extracts, we have tried using the capillary electrode described by Hakon Lund and myself (*Annales de Chimie, l.c.*) and shown in Fig. 1.

The electrode is a thin glass tube drawn out into a point. A long, thin platinum wire is used as electrode, and this is melted into a glass tube in the usual way. The two glass tubes are fastened together with a piece of heavy rubber tubing or a rubber cork. A small quantity of quinhydrone is mixed with the liquid to be examined, and by turning the two tubes in relation to each other, a couple of drops are sucked into the pointed end of the tube, so that 1–2 mm. of the platinum wire are moistened by the liquid. An element is then formed by means of a standard electrode, a glass with a saturated solution of KCl and the capillary electrode, the point of the latter being slightly dipped into the solution of potassium chloride, as shown in the figure. The potential may be measured at once, as otherwise diffusion will change the composition of the solution.

In order to investigate the application of this quinhydrone electrode on soil extracts we have determined the pH of extracts of seven different soil samples both colorimetrically (with bromthymol blue and α-naphtholphthalein) and electrometrically with the capillary electrode. The results are shown in Table II, where π is the potential of the quinhydrone standard electrode in relation to that of the capillary electrode.

Table II.

Soil extract	pH colorim.	π	pH electrom.
No. 1	7·1	0·296	7·2
2	7·4	0·326	7·7
3	7·7	0·330	7·8
4	7·3	0·304	7·3
5	7·2	0·302	7·3
6	7·4	0·310	7·4
7	7·0	0·286	7·0

We find that the agreement is entirely satisfactory, for the average variation between two determinations of pH in the same sample is 0·07.

Finally the pH was measured in a mixture of soil and water, and we examined both the same seven soil samples and four others.

In this investigation we used both ordinary electrode vessels and another, even simpler arrangement, in which the electrode vessels of the calomel electrode type are replaced by ordinary test-tubes. The method of procedure is then as follows:

Five gm. of the soil sample are shaken for a short time in a test-tube with 20 c.c. of boiled, distilled water and some centigrams of quinhydrone. The platinum electrode is placed in the mixture of soil, water and quinhydrone and a cell is formed by hanging the standard electrode by its syphon-tube on the edge of the test-tube, so that the end of the syphon-tube dips a few millimetres into the liquid in the test-tube. Fig. 2 shows the arrangement. The measurement of the potential has to be made at once.
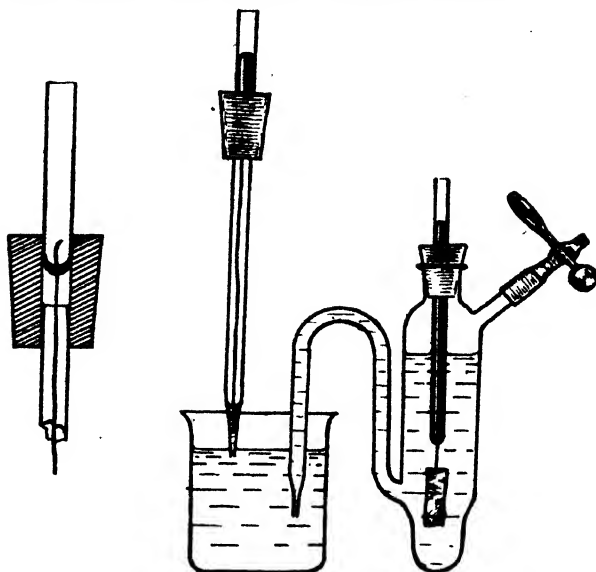
Fig. 1.
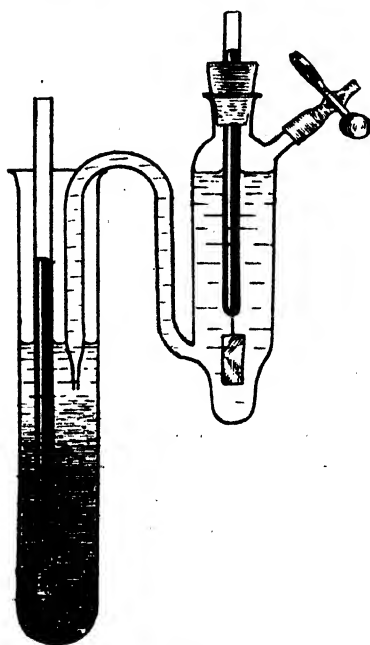
Fig. 2.

Table III gives the result of measurements of the 11 mentioned mixtures of soil[1] and water, both with test-tubes as electrode vessels and with ordinary electrode vessels of the calomel electrode type.

Table III. *Quinhydrone in mixtures of soil and water.*

Soil sample	Ordinary electrode		Test-tube electrode	
	π	pH	π	pH
No. 1	0·292	7·1	0·288	7·0
2	0·335	7·8	0·337	7·9
3	0·352	8·1	0·345	8·0
4	0·336	7·9	0·325	7·7
5	0·284	7·0	0·285	7·0
6	0·343	8·0	0·338	7·9
7	0·362	8·3	0·351	8·1
8	0·070	3·3	0·064	3·1
9	0·222	5·9	0·231	6·0
10	0·076	3·4	0·070	3·3
11	0·078	3·4	0·079	3·4

The agreement between the two series is quite satisfactory, but the values for pH are generally lower when the measurements are made with the test-tube as electrode vessel. It is possible that this is due to a diffusion of acid from the standard electrode and therefore it may be advisable to bend the point of the syphon-tube of the standard electrode upwards, because the liquid of the standard electrode has a higher specific gravity than the liquid in the other electrode. The practical advantage of the use of the test-tubes instead of electrode vessels of more complicated form is so evident for soil investigations on a large scale that a further development of this detail seems desirable. In the State Laboratory for Plant Culture it has been found advantageous to establish the connection between the standard electrode and the test-tube electrode not directly but through a vessel with potassium chloride solution and siphon-tube filled with a stiffened solution of agar and potassium chloride and dipping one branch in the vessel with potassium chloride and the other branch in the test-tube electrode. M. Harald R. Christensen and Mr Tovborg Jensen will describe the arrangement in a special paper.

A few practical hints on the treatment of the platinum electrodes remain to be made. If the electrodes require thorough cleaning, they should be treated with a hot mixture of chromic acid and strong sulphuric acid, then washed with distilled water and heated to glowing-point over an alcohol vapour lamp or a benzine blow-lamp, but *not in gas flame.*

[1] Samples 1–7 are the same as those of which the pH values are given in Table III. We find that the extracts show on an average a lower value for pH than the corresponding mixture of soil and water.

However, during the daily work in examining series of soil samples, rinsing with distilled water, when going from one sample to another, may be sufficient.

The formula by means of which the value of *p*H is calculated from the measured potential is exact at 18° C. But as it is not to be taken for granted, that a soil sample represents the hydrogen-ion concentration of the soil with greater accuracy than 0·1–0·2 in *p*H, it is permissible to work at temperatures which deviate some few degrees from 18° C.

The quinhydrone is conveniently prepared as follows: 100 gm. ferric ammonium alum are dissolved in 300 c.c. water at ca. 65° and this solution is poured into a warm solution of 25 gm. hydroquinone in 300 c.c. water. The quinhydrone precipitates in fine dark needles. The mixture is cooled in ice and filtered by suction and the precipitate then washed four or five times with cold water. Yield 15–16 gm. The preparation may contain a slight trace of iron, which is without serious effect.

When I had found that the quinhydrone electrode could be used so easily and rapidly in measuring hydrogen-ion concentrations in soils, it seemed important to try out the method on a larger scale. I therefore went to the State Laboratory for Plant Culture (Statens Planteavls Laboratorium) in Lyngby, Denmark, where the Director, Mr Harald R. Christensen, showed much interest in the matter and undertook the examination of a large number of samples of various kinds of soils. The measurements were made by his assistant, Mr Tovborg Jensen. By using the ordinary hydrogen electrode at the same time as the quinhydrone electrode for making *p*H determinations, it was possible to compare the accuracy of the two methods. I cannot express my gratitude warmly enough to both Mr Harald R. Christensen and Mr Tovborg Jensen for the interest they have shown in the question and for putting the following report of their experiments at my disposal.

APPENDIX:—*Comparative determinations of hydrogen-ion concentrations in soils, made at The State Laboratory for Plant Culture, Lyngby, Denmark.*

The determinations were made by means of the usual hydrogen electrode, as well as by means of the quinhydrone electrode. Mixtures of 5 gm. soil and 20 gm. boiled distilled water were shaken in a test-tube with some few centigrams of quinhydrone and poured into vessels of the type used for calomel electrodes. As comparison electrode the quinhydrone standard electrode described by Veibel was used. Every morning before beginning measurements the platinum plates of the quinhydrone electrodes were rinsed with distilled water and heated in the flame of a benzene blow-lamp. The potentials were measured some five minutes after inserting the platinum in the mixture. Table IV contains the values of *p*H found with the hydrogen electrode (H_2) and with the quinhydrone electrode (Q) on the examination of 75 different soil samples.

Table IV.

(a) Sandy soils.

H₂	Q	H₂	Q	H₂	Q	H₂	Q
8·28	8·28	8·00	8·18	7·90	7·95	7·88	7·82
7·84	7·90	7·89	8·08	7·74	6·73*	7·60	7·64
7·26	7·34	6·94	6·96	6·90	6·86	6·84	6·90
6·82	6·74	6·80	6·78	6·80	6·87	6·70	6·59
6·68	6·62	6·56	6·62	6·50	6·37	6·36	6·37
6·30	6·62*	6·30	6·36	6·15	6·14	6·04	6·20
6·02	6·10	6·02	5·92	5·96	5·92	5·82	5·90
5·50	5·64	5·40	5·96*	5·39	6·56*	5·32	5·42

(b) Loam soils.

H₂	Q	H₂	Q	H₂	Q	H₂	Q
8·22	8·20	8·16	8·22	7·96	8·10	7·88	7·93
7·85	8·00	7·78	7·94	7·72	7·64	7·60	7·48
7·48	7·50	7·32	7·52	7·31	7·39	7·24	7·26
7·12	7·04	7·10	7·04	7·08	6·98	6·94	7·10
6·70	6·80	6·70	6·74	6·60	6·76	6·38	6·47
6·30	6·38	6·30	6·18	6·22	7·28*	6·21	6·35
6·20	6·53*	6·15	6·53*	6·00	6·10	5·86	5·93
5·82	5·79	5·44	5·46	5·28	5·32	—	—

(c) Humus soils.

H₂	Q	H₂	Q	H₂	Q	H₂	Q
7·84	7·71	7·66	7·49	7·46	7·46	6·87	6·76
6·68	6·76	5·96	6·02	5·96	6·04	5·68	5·71
5·54	5·60	4·70	4·78	4·76	4·70	4·23	4·25

The accordance between the two series of figures is very good. In most cases the agreement is within 0·1 in the value of pH, and only in seven cases (marked with an asterisk) do the deviations exceed 0·2, which may be considered as the precision needed in the determinations of pH in soil samples. In five of the seven cases the determinations were repeated (Table V). Comparison of the first and the second series of measurements seems to show, that the differences in the first series are due to sampling and other errors, which may easily be avoided.

Table V.

First determinations		Second determinations	
H₂	Q	H₂	Q
7·74	6·73	6·34	6·38
6·30	6·62	6·38	6·65
5·40	5·96	6·02	6·04
6·22	7·28	6·36	6·44
6·15	6·53	6·45	6·50

Altogether some 200 comparisons have been made with results similar to those recorded here. Measurements on 500 samples have been made with a special form of the test-tube electrode described in the first part of this paper and will be discussed in a separate paper.

FIELD EXPERIMENTS IN ELECTRO-CULTURE[1].

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ATTEMPTS to increase the growth of plants by means of electricity date back to the 18th century, Mambray at Edinburgh in 1746 being apparently the first to conduct experiments. Various methods of electrification have been employed; use has been made of the electricity of the atmosphere and of a charged spray of water, and currents have been sent through the soil. Good accounts of earlier experiments are given by Grandeau(6), Giglioli(5), and Priestley(14), see also Jørgensen and Stiles(10). Owing to the work of Lemström, attention has of late years been concentrated mainly on the electrification of crops by means of a discharge from thin wires placed above the crop. Lemström, a professor of physics at Helsingfors, when on a visit to the northern polar regions came to the belief that the rapid growth of vegetation during the short arctic summer was to be ascribed to special electrical conditions of the atmosphere in these high latitudes[2]. On his return to Helsingfors he attempted to reproduce these assumed conditions by increasing the atmospheric current, which normally passes from the air to the plant, by the use of wires placed above the crop and charged to a high potential by means of an influence machine. The results of his experiments were published in book form and translated into English in 1904(12).

Lemström claimed to have obtained from electrification an increased yield in many cases and with many different plants. His results, however, though suggestive failed to carry conviction to most agricultural workers since he did not appear to be familiar with the large experimental error

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[1] The experimental results here described have been incorporated in the reports of the Electro-Culture Committee of the Ministry of Agriculture and Fisheries, and a brief statement of the results has appeared in the *Journal of the Ministry of Agriculture*, xxix. 792, 1922.

[2] The claim that in the polar regions the atmospheric current is of higher intensity than in lower latitudes is still unproven.

implicit in the field plot method, nor was the previous history of his experimental ground usually known.

Using Lemström's method, which is often referred to as that of the "overhead discharge," a number of field experiments have been carried out, for example by Priestley[14, 14a, 15], Hendrick[7], Lodge[13], and Newman (see 13 and 14a) in this country and by other workers elsewhere[1, 5, 11, 17, 18]; Mr I. Jørgensen and the author[3, 8] have also published the results of single field experiments carried out during the years 1915 and 1916. The results of these experiments have, however, been very variable, and failed to give full proof of a favourable effect of the electric discharge. Most of them were isolated field experiments lasting but a single season, and the electrical conditions were very imperfectly known, measurements of the intensity of the current passing from the wires being usually lacking. Failure might thus be due to too high or too low a discharge; in fact such experiments might be compared to a manurial experiment in which a fertiliser is applied in unknown quantities. The absence of appropriate physical measurements is of course particularly unfortunate since without them it is impossible to reproduce in a later experiment the electrical conditions of an earlier one.

It became clear that satisfactory evidence as to the value of the overhead discharge could be gained only from a series of field experiments carried out with appropriate physical measurements and for a number of years. Furthermore it was realised that although in the present state of our knowledge some success might be obtained in field experiments, yet any such success would be of a haphazard nature until further knowledge, both of the physiological conditions under which the discharge should be given and of the physiological effect of the discharge, were available. Without such physiological knowledge field experiments must be largely of an empirical nature and carried out under arbitrarily chosen conditions. On this ground pot-culture experiments were started in 1918, for owing to their much smaller experimental error these cultures yield trustworthy physiological data much more rapidly than field experiments. Laboratory experiments on the effect of minute electric currents on growth were also undertaken. The results of the pot-culture experiments are embodied in the paper which follows this[4]; the first results of the laboratory work have been published elsewhere[2].

In all the experiments from 1917 to 1920 here described not only has the potential of the discharging wire been determined, but the intensity of the current passing from the overhead installation has been carefully measured, usually by a milliammeter such as is used for

X-ray work[1]. The instrument was at first placed in the return (earth) circuit. In this position, however, it is liable to give too high readings, so later it was placed in the high tension circuit.

Another respect in which the experiments here described differ from all previous work in electro-culture is their extent and duration and their association with experiments in pot-culture and laboratory investigations. No less than 16 field experiments have been undertaken, spread out over four years and carried out at three different centres. By repetition of the experiments the uncertainty due to the large experimental error inherent in field trials can be avoided[2]. The continuance of the experiments over a number of years and at a number of centres allows also for the effect of different seasons and of different soil conditions.

In the earlier experiments of this four year period the type of apparatus used by previous workers was employed, the high tension current being obtained by the use of a mercury interrupter and an induction coil, with rectification of the current by the use of Lodge valves. In the later experiments apparatus more in consonance with electrical engineering practice was used at the Rothamsted Experimental Station and the Harper Adams Agricultural College, namely, an A.C. dynamo (50 cycles) and an oil immersed transformer giving 60,000 volts, while rectification was obtained mechanically by means of a rotating disc rectifier (Newton and Wright type) fixed on an extension of the spindle of the dynamo.

The field installation consisted of cables of stout galvanised iron, or stranded steel, wire placed on two sides of the plots. These cables were supported at a height of about 7 feet above the ground by disc insulators, three in series, fixed to wooden posts. Between the cables ran the fine discharging wires which were usually of fine galvanised steel (0·35 mm. diam.). This low type of installation though not satisfactory for actual agricultural practice is the most suitable for experimental work since with high wires the spread of the discharge is very great as Jørgensen and Priestley(9) have shown. With wires at a low level the control plots can be kept nearer the electrified plots than with wires at a height of 15 or 18 ft., and so the chances of difference in soil conditions between the two areas can be reduced.

The wires were in all cases charged positively.

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[1] It is found most satisfactory to keep this ammeter short-circuited when not in use as the sparking employed in voltage measurements is liable to damage the instrument.

[2] The chequer-board system of plots, which is of such value in ordinary field trials, is impossible in electro-culture owing to the lateral spread of the ions of the air.

The intensity and duration of the current to be employed in these experiments, and the period of the discharge were arbitrarily chosen. The current strength was at the rate of 0·5 to 1 milliamp. per acre given for two periods during the day, *i.e.* about 3 to 4 hours in the morning, and about 3 to 4 hours in the afternoon. The hot, middle period of the day was avoided in accordance with Lemström's view that the application of the discharge is then not advantageous; this view however requires more definite experimental support.

EXPERIMENTS OF THE YEAR 1917.

Experiment at Lincluden, Dumfriesshire.

Experiment with Oats (var. Potato). This experiment was similar to those of 1915 and 1916, a description of which has already been published (3, 8). The experimental and control areas were each of one acre (146·75 × 33 yds.) with their long sides parallel and a space of 40 yards between. The control plot lay s.w. of the electrified plot. The uniformity of the soil of the field was not all that could be desired.

Apparatus. This consisted of a mercury interrupter working on a 60 volt current, an induction coil with Lodge valves employed for rectification.

Field Installation. Tinned steel wires (s.w.g. 30) 18 ft. apart were stretched across the experimental area, the height being 6 ft. in the middle and 7 ft. at the ends. The wires were charged positively.

Current. A milliammeter was placed in the return (earth) circuit[1]. The readings at the beginning of the experiment were about 0·5 milliamp. rising to nearly 1 milliamp. towards the end of the experiment.

Period of Discharge. The total period during which the discharge was given was 1060 hours; the discharge was usually started at 6–8 a.m. and continued until about 6 p.m.; it was discontinued in wet weather. Electrification was continued from May 15th till August 27th. The grain was sown on April 23rd and harvested at the end of August. Owing to a breakdown in the binding mechanism of the harvester the straw yields cannot be given. Each area was divided into three equal plots which were harvested separately.

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[1] This is not a satisfactory experimental arrangement, and in all the experiments of later years the ammeter was placed in the high tension circuit, *i.e.* between the induction coil, or transformer, and the installation.

Crop Results.

Yields per acre

Electrified	Controls
E. I　54·8 bushels (42 lbs.)	C. I　48·9 bushels
E. II　42·2　,,	C. II　44·9　,,
E. III　36·9　,,	C. III　38·1　,,

Difference between mean of electrified and mean of control plots　+2 %

The difference is too small to be significant. The seasonal conditions were not very favourable as at times the crop suffered severely from drought; from June 25th to July 9th the rainfall was only 0·1 in. The winds also were unusual, as on more than half the days during which the experiment was carried on the winds were N.NE., E. or S.E.; the discharge was thus carried to a certain extent over the control area.

Experiment at Rothamsted Experimental Station.

Experiment with Barley. A plot of land about 1/40th acre in the neighbourhood of the laboratories was divided into a control and an electrified area by "earthed" galvanised wire netting (partly ½-inch and partly 1-inch mesh) 8 ft. high so as to prevent the spread of the discharge to the control area. The area of the control plot was 3 per cent. greater than that of the experimental plot.

Apparatus. This was similar to that of the experiment at Lincluden described above.

Installation. Three tinned steel wires (S.W.G. 30) 32 ft. long and about 6 ft. apart were stretched over a height of 7 ft.

Current. A microammeter was placed in the earth circuit. The reading varied very much, but was about 10–15 microamps.

Period of Discharge. The discharge was started on May 31st and continued until September 8th, the daily period being about 15 hrs. (4–12 a.m., 1–8 p.m.). Owing to special conditions the crop could not be sown till May 25th. It was harvested on Sept. 8th before it was fully ripe to avoid depredations by birds.

Crop Result.

Yield per acre

	Electrified	Control
Total yield ...	29·5 cwt.	24·1 cwt.
Grain　...	17·8 bushels (56 lbs.)	13·1 bushels
Difference between yield of electrified and control plots　...　...　...　...}	Straw plus grain +22 % Grain ...　... +36 %	

By June 19th, *i.e.* after 20 days' electrification, there was a difference between the crops of the two areas which was noticeable to a casual observer. The plants on the experimental area were taller and appeared to be of a deeper green colour. Later on the visual difference between the two crops was less marked. The final weights show a marked difference in favour of the electrified which may be taken as significant.

EXPERIMENTS OF THE YEAR 1918.

Experiment at Lincluden, Dumfriesshire.

Experiment with Oats (var. Potato). The electrified and control areas ran parallel about 40 yards apart, and the control area lay s.w. of the electrified. The electrified area was 100 yards by 40 yards, *i.e.* 4000 sq. yards (of which 3000 sq. yards were harvested), and the control area was 900 sq. yards (8 by 112·5 yards). The electrified and control areas were each divided into three equal sections which were harvested separately.

Apparatus. This consisted of a mercury interrupter, supplied with direct current at a voltage of 60, an induction coil, and three Lodge valves in series.

Wiring. A stranded steel cable supported on high tension insulators was fixed at a height of 7 ft. at each side of the electrified area and fine galvanised steel wires (gauge 29) spanned the 40 yards between the cables. The wires were 15 ft., 10 ft., and 5 ft. apart respectively on the three sections, the total length of wire being 1440 yards. The aerial installation was made positive.

Current. A milliammeter (which was kept short circuited except when readings were being taken) was placed on the high tension side of the field circuit and a recording microammeter in the return circuit. The current, which was controlled by altering the voltage, varied between 0·5 and 0·9 milliamp., but was usually about 0·8 milliamp. If the discharge currents are proportional to the number of wires this should give a discharge at the rate of 0·5, 0·75, and 1·5 milliamps. per acre over the three sections.

Period of Discharge. The discharge was given during 704 hours altogether, starting at 5.30 a.m. or 6.30 a.m. (G.M.T.) in the morning and running till about 10.30 a.m. and then again for about 3 hours in the afternoon, stopping at about 5.30 p.m. The warmer middle hours of the day were avoided, and the discharge was not given during hot, droughty weather nor during rain.

The sowing of the field was finished on March 25th, the crop appeared above ground on April 10th, but the discharge was not started until April 19th, owing to the dry condition of the ground; it was continued till August 11th. The crop was cut on August 15th and 16th. The yields were as follows:

Crop Results.

	Electrified (Yield per acre)		Control (Yield per acre)
	E. I (wires 5 ft. apart)		C. I
Grain ...	3170 lbs. = 75·5 bushels	Grain ...	2355 lbs. = 56·1 bushels
Straw ...	51·3 cwt.	Straw ...	45·3 cwt.
	E. II (wires 10 ft. apart)		C. II
Grain ...	3567 lbs. = 84·9 bushels	Grain ...	2452 lbs. = 58·4 bushels
Straw ...	63·7 cwt.	Straw ...	46·5 cwt.
	E. III (wires 15 ft. apart)		C. III
Grain ...	3378 lbs. = 80·4 bushels	Grain ...	1946 lbs. = 46·3 bushels
Straw ...	53·7 cwt.	Straw ...	42·6 cwt.
	Mean		Mean
Grain ...	80·3 bushels per acre	Grain ...	53·6 bushels per acre
Straw ...	56·2 cwt. per acre	Straw ...	44·8 cwt. per acre

Difference between mean yields of electrified and control plots ... Grain ... +50 % Straw ... +25 %

The nitrogen and organic matter of the six plots were estimated in the usual way with the following results:

	Nitrogen per cent.		Organic matter	
	Surface	Subsoil	Surface	Subsoil
E. I	1·33	0·22	10·2	7·3
C. I	0·31	0·17	8·4	6·1
E. II	0·36	0·27	10·0	8·6
C. II	0·34	0·20	10·0	7·3
E. III	0·37	0·25	9·7	8·4
C. III	0·34	0·20	10·0	7·7

These estimations show that the electrified plots are somewhat richer in nitrogen than the control plots, a difference which reduces the value of the crop results. The differences in crop yield are however too large to be explained by the nitrogen differences.

Rothamsted Experimental Station.

Experiment with Barley (Burton). The total electrified area was 2·1 acres, the three plots harvested being each 2/3rds of an acre. The three control plots lay s. of the electrified area and were each 1/10th acre.

Apparatus. This consisted of a petrol-driven engine and dynamo ("Delco") giving alternating current, and a wax impregnated transformer. The current was rectified by means of a Newton and Wright disc rectifier.

Field Installation. This was of the usual type. Thin galvanised steel wires (s.w.g. 29) were stretched between cables placed at a height of 7¼ ft. at the sides of the area. The cables were insulated by means of high tension disc insulators, three in series. The fine wires were 5 ft., 10 ft. and 15 ft. apart respectively over the three electrified areas. There was a considerable sag in the middle of the plot. The total length of fine wire was 2·1 miles.

Current. A milliammeter was placed in the earth circuit and the current was kept at the rate of 1 milliamp. per acre by controlling the voltage in the primary of the transformer. The voltage varied from 35,000–60,000 (crest value) decreasing as the crop grew up and varying with weather conditions.

Period of Discharge. The discharge was supplied from April 26th to August 16th, the total number of hours being 643. The daily period of discharge was from 7–11 a.m. and from 2–6 p.m. (G.M.T.); it was usually continued even during rain.

The crop was harvested on Sept. 6th and 7th.

Crop Results.

Yields per acre

	Electrified			Control	
	Grain	Straw		Grain	Straw
E. I	44·7 bushels	22·3 cwt.	C. I	36·4 bushels	21·6 cwt.
E. II	47·4 ,,	25·0 ,,	C. II	52·7 ,,	28·5 ,,
E. III	40·4 ,,	24·4 ,,	C. III	36·3 ,,	21·8 ,,
Mean ... 46·2	,,	23·9 ,,		41·8 ,,	24·0 ,,

Difference between mean yield of ⎱ Grain + 10 %
electrified and control plots ... ⎰ Straw – 0·5 %

This result by itself is too small to be significant. An examination of the yield of the various plots shows that the value of the experiment has been markedly reduced by the plot C. II which gives a yield 45 per cent. higher than either of the other plots. If this plot be neglected the electrified area shows a yield in grain 24 per cent. greater than that of the controls.

EXPERIMENTS OF THE YEAR 1919.

The year was an unfavourable one for field experiments owing to the early wet period which delayed the planting of spring crops and the drought which occurred later. The effect of the drought is well seen in the low yields obtained; that at the Harper Adams Agricultural College is considerably below a normal yield, and the highest yield at Lincluden

is 53 bushels as compared with the 85 bushels obtained last year on the same field.

In all the three sets of field experiments of this year a wire screen was placed close to the control areas. This improves the experimental conditions from the electrical aspect by protecting the controls from stray currents, but from the agricultural aspect it is less satisfactory since it gives the control artificial protection from the wind.

Experiment at Lincluden.

Experiment with Oats (var. Potato). This experiment was conducted on the same field as that of 1918. A dressing of 3 cwt. superphosphate, 1 cwt. of sulphate of ammonia, and 2 cwt. of bone flour per acre was given. The control and electrified areas were each about 1/3rd acre and they ran at right angles to the plots used last year. The control lay s.e. of the electrified area and was protected by a screen of wire netting (1-in. mesh) 9 ft. high, which stood between the areas at a distance of 6 ft. from the control area. The areas were harvested in three equal sections.

Apparatus. This was the same as in the year 1918.

Wiring. The installation was similar to that of 1918, but the thin discharge wires were 5 ft. apart over one-half of the area and 10 ft. apart over the other half.

Current. The arrangements for measuring the current were the same as last year. The current given was about 0·25 milliamp., which should give 0·5 and 1·0 milliamp. per acre respectively over the two halves of the plot on the view that the discharge received by the crop is proportional to the number of wires per unit area.

Period of Discharge. The discharge was given for 710 hours running from April 21st to August 11th. The daily periods during which the discharge was given were the same as in 1918.

The sowing of the field was finished on April 4th and the crop was cut on August 12th.

Crop Results.

Electrified (Yield per acre)		Control (Yield per acre)	
E. I (wires 5 ft. apart)		C. I	
Grain ...	36·6 bushels	Grain ...	45·2 bushels
Straw ...	18·2 cwt.	Straw ...	27·9 cwt.
E. II (wires 5 ft. and 10 ft. apart)		C. II	
Grain ...	45·1 bushels	Grain ...	43·8 bushels
Straw ...	28·1 cwt.	Straw ...	27·9 cwt.

	Electrified (Yield per acre)		Control (Yield per acre)	
	E. III (wires 10 ft. apart)		C. III	
Grain ...	53·3 bushels		Grain ... 28·9 bushels	
Straw ...	36·4 cwt.		Straw ... 16·2 cwt.	

	Mean of E. I, E. II and E. III	Mean of E. II and E. III	Mean of C. I, C. II and C. III	Mean of C. II and C. III
Grain (bushels per acre)	45·0	49·2	39·3	36·4
Straw (cwt. per acre)	27·6	32·3	24·0	22·1

Difference between mean grain yield of electrified and control plots   +14 %

  „   „   straw   „   „   „   +15 %

Difference between mean grain yields of Plots E. II and E. III and
of Plots C. II and C. III   ...   .   +35 %

Difference between mean straw yields of Plots E. II and E. III and
of Plots C. II and C. III   ...   .   +46 %

The results of the nitrogen estimations of the soil of the Lincluden plots, which were carried out at the Rothamsted Experimental Station, are given below:

	Surface Percentage of N in dry matter		Subsoil Percentage of N in dry matter	
April 1919				
C. I	0·37 ⎫		0·24 ⎫	
C. II	0·39 ⎬ Av. =0·37		0·25 ⎬ Av. =0·22	
C. III	0·36 ⎭		0·18 ⎭	
E. I	0·35 ⎫		0·23 ⎫	
E. II	0·35 ⎬ Av. =0·35		0·23 ⎬ Av. =0·23	
E. III	0·36 ⎭		0·22 ⎭	
September 1919				
C. I	0·36 ⎫		0·26 ⎫	
C. II	0·39 ⎬ Av. =0·37		0·27 ⎬ Av. =0·22	
C. III	0·37 ⎭		0·13 ⎭	
E. I	0·36 ⎫		0·24 ⎫	
E. II	0·38 ⎬ Av. =0·37		0·25 ⎬ Av. =0·24	
E. III	0·36 ⎭		0·24 ⎭	

The little difference between the nitrogen content of the two sets is in favour of the controls. The superiority of the crop on the electrified area must then be due to some factor other than nitrogen supply.

The difference in crop yield observed is comparatively small, but in assessing its value the drought, which was particularly severe in the Dumfries district, must be borne in mind. Also the wire screen protected the control from northerly winds.

The low yield of E. I is almost certainly due to the snow blizzard of April 27th, which did obvious damage to that area, while the corresponding control area, C. I, was protected by the snow which, collecting on the wire netting, formed an almost solid screen.

The mean results have therefore been calculated both on the basis of the whole areas and also after exclusion of areas E. I and C. I. Owing to the special conditions to which E. I and C. I were subjected by the

snowstorm of April the second result is probably the more trustworthy; it has accordingly been chosen for inclusion in the summary given on p. 261.

In 1918 the plot E. III gave a yield only 5 per cent. higher than C. III when neither was electrified; in 1919 when E. III was electrified it gave a yield 84 per cent. higher than the control.

The results of the Lincluden experiment may be considered significant when taken in connection with the other positive results of this year and last.

Experiments at Rothamsted Experimental Station.

1. *Experiment with Clover-Hay.* This experiment was undertaken in the field (Foster's Field) used for the barley experiment of 1918, "seeds" having been sown in 1918 with the barley. Plots about 1/3rd of an acre were selected from the C. I and C. III plots which had given equal yields last year. After the first crop was cut a second control was marked out on the electrified area of last year, and the first control was surrounded with wire netting, as there was some evidence that hares and rabbits had been visiting this area.

The apparatus and wiring was similar to that of the other experiments, the wires being 10 ft. apart.

Current. As three installations were supplied from the same transformer the intensity of the discharge current could not be altered independently. The voltage was kept at such a height as to give a rate of discharge of about 0·5 milliamp. to the winter wheat area. Such a voltage gave a rather higher rate of discharge to the clover; it started at about 0·6 milliamp. per acre and rose to 1·6 milliamps. per acre. Electrification was begun on April 12th and continued until September 10th. The first crop of clover was electrified for 354 hours and the second crop for 440 hours.

The following results were obtained:

First Crop.

Control I = 23·1 cwt. per acre   Electrified = 34·8 cwt. per acre
Difference between yield of electrified and control areas   +50 %

Second Crop.

Control I = 14·0 cwt. per ac.   Control II = 11·6 cwt. per ac.   Electrified = 17·1 cwt. per ac.
Increase over mean of controls = 34 %

The results of the nitrogen determination are given below; the samples were taken on Feb. 23rd, 1920.

Percentage of N in dry matter (surface soil)

C. I   0·16   C. II   0·15   E.   0·15

The differences in nitrogen content are very slight and are somewhat in favour of the control plot C. I.

The "stand" of clover was somewhat irregular in various parts of the field, but the differences in favour of the electrified area reach a significant value.

2. *Experiment with Winter Wheat.* This experiment was undertaken on Great Knott field sown with a winter wheat (Red Standard) in early November, but electrification was not started until April 12th. The electrified and control areas were each 1 acre, the control area lying about 110 yards to the south-west of the electrified area.

Apparatus. This consisted, as in 1918, of a petrol-driven "Delco" set, with a dry transformer and a Newton and Wright disc rectifier.

Field Installation. This was similar to that at Lincluden and that of 1918. Over half the area (E. I) the wires were 10 ft. apart, and over the other half (E. II) the wires were 5 ft. apart.

Current. The current varied between 0·5 and 1·0 milliamp. per acre. This installation, that of the clover-grass experiment just described and that of the next experiment, were all supplied from the same transformer. By the use of three separate out-going circuits and ammeters in two of them and in the return circuit, the discharge from each installation could be measured. As the intensity of the discharge was controlled by the voltage it could only be regulated in one installation at a time; that of the winter wheat was usually kept at a fixed intensity.

Period of Discharge. The current was started on April 12th and continued to August 19th, the total period of the discharge being 854 hours. The discharge was given usually from 7–11 a.m. and from 4–6 p.m. (G.M.T.).

The crop was sown November 9th–11th, and harvested in September.

Crop Results.

	Electrified (Yield per acre)		Control (Yield per acre)
	E. I (wires 10 ft. apart)		C. I
Grain ...	21·4 bushels (60 lbs.)	Grain ...	14·3 bushels
Straw ...	11·9 cwt.	Straw ...	8·2 cwt.
	E. II (wires 5 ft. apart)		C. II
Grain ...	22·3 bushels	Grain ...	17·4 bushels
Straw ...	13·5 cwt.	Straw ...	9·7 cwt.
	Mean		Mean
Grain ...	21·9 bushels	Grain ...	15·9 bushels
Straw ...	12·7 cwt.	Straw ...	9·0 cwt.

Mean increase of grain $= 38 \%$
    ,,   ,,   straw $= 41 \%$

The results of the nitrogen estimations of the soil of these plots are given below; the samples were taken on Feb. 3rd, 1920.

Percentage of N in dry matter (surface soil)

C. I	0·16	E. I	0·16
C. II	0·17	E. II	0·16

The agreement is seen to be satisfactorily close.

The positive result obtained with this crop may be taken as definitely significant. It is the first time in this set of experiments that a *winter* cereal crop has been subjected to the discharge. It indicates that such a crop can profit from the discharge even though the application is delayed until the spring.

3. *Experiment with Spring-Sown Wheat.* This experiment with wheat (Red Standard) was undertaken on the same field as the previous experiment with winter-sown wheat. The electrified and control areas were each 1 acre in extent, the electrified being divided into two plots and the control into three plots.

Apparatus and Installation. These were the same as in the experiment with winter wheat described above, the discharge wires above E. III being 5 ft. apart and those above E. IV 10 ft. apart.

Current. The current varied from 0·1 to 1·5 milliamps. per acre, but was usually about 0·75 milliamp.

Period of Discharge. The discharge was given for 940 hours running from April 18th to September 8th. The daily periods during which the discharge was given were the same as in the previous experiment.

The sowing was finished on March 26th, and the crop was cut in September.

Crop Results.

Electrified (Yield per acre)		Control (Yield per acre)	
E. III (wires 5 ft. apart)		C. III	
Grain ...	7·6 bushels (60 lbs.)	Grain ...	10·0 bushels
Straw ...	7·4 cwt.	Straw ...	8·3 cwt.
E. IV (wires 10 ft. apart)		C. IV	
Grain ...	7·3 bushels	Grain ...	7·9 bushels
Straw ...	8·5 cwt.	Straw ...	8·1 cwt.
		C. V	
		Grain ...	6·3 bushels
		Straw ...	8·3 cwt.
Mean		Mean	
Grain ...	7·5 bushels	Grain ...	8·0 bushels
Straw ...	8·0 cwt.	Straw ...	8·2 cwt.

Difference between means of electrified and control areas:

Grain = − 8 %   Straw = − 3 %

The results of the nitrogen estimations of these plots are given below; the samples were taken on Feb. 3rd, 1920.

Percentage of N in dry matter (surface soil)

C. III	0·17	E. III	0·17
C. IV	0·16	E. IV	0·13
C. V	0·14		

This experiment was carried out under very unsatisfactory conditions. A wet period delayed sowing until very late (March 26th), and soon after the crop was up and before it was well established a drought set in. The poorness of the crop is shown by the mean yield of grain, which is only about a quarter of an average yield. The negative result cannot, under the circumstances, be considered as of any significance.

This experiment is the first of the series of agricultural experiments carried out since 1915 which has failed to give a positive result.

Experiment at the Harper Adams Agricultural College.

Experiment with Oats (var. Crown). This experiment was conducted in a large flat field of a good loam with a somewhat sandy subsoil. The electrified and control areas ran parallel about 75 yards apart and the electrified area lay N.E. of the control area. The control area measured 110 yds. by 44 yds., *i.e.* 1 acre, while the electrified measured 110 yds. by 66 yds., *i.e.* 1½ acres. Each area was divided into three equal sections (half an acre), and since the 5 ft. and 10 ft. wires met in the middle of the central section of the electrified area, this section was again subdivided into two equal halves, E. II (*a*) and E. II (*b*).

Apparatus. Current (100 volts D.C.) was available from the small electric lighting installation of the College. The apparatus employed consisted of a small 2 H.P. motor (100 volts) coupled to a 1 K.V.A. A.C. dynamo (140 volts) which bore on an extension of its axle a Newton and Wright disc rectifier. An oil-cooled transformer (1 K.V.A.), giving a voltage up to 60,000, was employed for the discharge current.

Field Installation. This was of the same type as those put up last year at Rothamsted and Lincluden. The cables were 7 ft. high at the ends and the fine stranded wires (gauge 29) were 5 ft. apart over plots E. I and E. II (*a*), and 10 ft. apart over E. II (*b*) and E. III. A screen of wire netting 8 ft. high was fixed between the two areas and at a distance of 8 ft. from the control area.

Current. A milliammeter was placed in the earth circuit and the discharge was kept steady at 1·1 milliamps. by altering the resistance in the primary circuit. Such a current should give a discharge at the rate of 0·5 and 1·0 milliamp. per acre over the areas under the 10 ft. and

5 ft. wires respectively. The voltage varied from about 30,000 to about 50,000 (crest value), decreasing as the crop grew up and also varying with weather conditions.

Period of Discharge. The discharge was applied from May 23rd to August 14th and for a total period of 456 hours. It was given usually from 7–10 (G.M.T.) in the morning and for three hours in the afternoon, usually from 4–7, or somewhat later. The field was sown on April 2nd and harvested on Aug. 21st and 22nd.

Crop Results.

	Yield of grain bushels (39 lbs.) per acre	Total produce cwt. per acre
E. I	47·0	45·5
E. II (a)	63·8	53·0
E. II (b)	50·8	54·0
E. III	60·2	60·0
Mean ...	55·4	Mean ... 53·4
C. I	53·6	46·5
C. II	48·2	47·25
C. III	59·6	53·75
Mean ...	53·8	Mean ... 49·2

Difference between mean yield of electrified and control plots:

Grain +3 %   Straw plus grain +9 %

Nitrogen estimations of the soil of these plots are given below:

	March 1919		October 1919	
	Surface	Subsoil	Surface	Subsoil
E. I	0·14	0·07	0·13	0·07
E. II	0·14 } Mean 0·14	0·08 } Mean 0·08	0·15 } Mean 0·14	0·07 } Mean 0·06
E. III	0·14	0·08	0·14	0·05
C. I	0·13	0·08	0·13	0·08
C. II	0·14 } Mean 0·14	0·07 } Mean 0·08	0·14 } Mean 0·14	0·07 } Mean 0·07
C. III	0·14	0·09	0·14	0·06

The agreement between the two sets of plots is seen to be very close.

The 3 per cent. difference in grain yield is too small to be significant when taken by itself. In connection with this result the abnormal nature of the seasonal conditions must be considered; the severity of such conditions is shown by the lowness of the yield. This variety of oats when last sown on this field gave a yield of 90 bushels per acre. The low yield in 1919 appears to have been mainly due to attack by frit fly, which was favoured by the dry season; to the same cause is probably to be attributed the want of uniformity in the yield of neighbouring plots. Also the protection of the control plots from wind which the screen afforded may possibly have introduced a new factor.

EXPERIMENTS OF THE YEAR 1920.

Experiments with Oats at Lincluden.

This experiment was conducted on exactly the same plots as those used in 1919, and the same crop was employed, but the variety was Swedish Victory. A dressing of 3 cwt. superphosphate, 1 cwt. ammonium sulphate, and 3 cwt. kainit was given. The three control and three electrified plots were each 1/9th of an acre in area and the control area lay S.E. of the electrified area.

Apparatus. This was the same as in 1918 and 1919.

Field Installation. The installation was similar to that of 1919, but the discharge wires were 5 ft. apart over the whole electrified area.

Current. The arrangements for measuring the current were the same as in 1919. The current was kept at about 0·25 milliamp., which should give a discharge of about 0·75 milliamp. per acre. The voltage (crest value) varied from about 14,000 to about 35,000 according to weather conditions.

Period of Discharge. The discharge was given generally for two daily periods (from about 6–11 a.m. and from about 5–8 p.m. (G.M.T.)) and for 911 hours in all, running from May 8th to August 29th.

The sowing of the field was finished in the first week of April, but owing to wet weather the fertiliser could not be put on until three weeks later. The crop was out on August 31st and threshed on September 27th. The wire screen between the control and electrified areas was not again used, as it was found in 1919 to act as a protecting wind screen. Observations made in 1920 with an electrometer showed that in spite of the absence of a screen the control area showed no indication of the discharge even when the wind was blowing over it from the electrified area.

The two sets of plots are arranged nearly parallel on ground which slopes down from plots I to plots III. It seems more satisfactory therefore to compare the plots in pairs, E. III with C. III, etc. The area E. I was unfortunately damaged by rabbits in the early stages of growth, so the plots E. I and C. I are neglected in the comparison. The plots were identical with those of 1919, and no fresh nitrogen estimations of the soil were therefore made.

The difference between the first set of plots is negative, but not significantly so; the difference between the second set of plots is very marked. Both the sets of plots are included in the summary set out on p. 261.

Crop Results.

	Electrified (Yield per acre)		Control (Yield per acre)
	E. I		C. I
Grain ...	36·2 bushels (42 lbs.)	Grain ...	44·8 bushels
Straw ...	30·4 cwt.	Straw ...	35·3 cwt.
	E. II		C. II
Grain ...	43·5 bushels	Grain ...	46·1 bushels
Straw ...	34·5 cwt.	Straw ...	38·8 cwt.
	E. III		C. III
Grain ...	51·8 bushels	Grain ...	33·0 bushels
Straw ...	42·0 cwt.	Straw ...	33·8 cwt.

Difference of yield between E. II and C. II: Grain −6 % Straw −12 %

„   „   „   E. III and C. III: Grain +57 % Straw +24 %

Field Experiments at Rothamsted Experimental Station.

1. *Experiment with Clover-Hay.* This experiment was carried out on Foster's Field and was a continuation of that of 1919, the "seeds" being carried on for a second year. The same plot as in 1919 was used as the electrified area, and the control plots were also similar.

Apparatus and Field Installation. The apparatus and installation were exactly the same as in 1919, the thin wires being 10 ft. apart. The same petrol-driven dynamo supplied current for this experiment and also for the barley and wheat experiments described below.

Current. As three installations were supplied from the same transformer the current could only be controlled in one, that over the winter wheat being selected. The discharge given to the clover-grass thus varied from 0·33 milliamp. per acre to 1·3 milliamps. per acre. The discharge wires were kept at a voltage which varied between 20,000 and 50,000.

Period of Discharge. Electrification was started on April 9th and continued until June 28th. The crop was electrified for 501 hours, the usual periods being 7–11 a.m. and 1–5 p.m. (G.M.T.). No second crop was taken from the field.

Crop Results.

Control I	...	...	...	24·0 cwt. per acre
Control II	...	...	...	23·0 „ „
Electrified	...	...	...	24·0 „ „

The difference between the yield of the electrified area and the mean of the control areas   ... +2 %

The results of the nitrogen determinations, which were made at the Rothamsted Experimental Station, are given below. The samples were taken on August 23rd.

Percentage of N in dry matter (surface soil): C. I = 0·16; E. = 0·16

The two areas C. I and E. are thus similar in respect of nitrogen content.

The difference in yield in favour of the electrified area is too small to be significant.

2. *Experiment with Barley.* This experiment with barley (Plumage × Archer) was carried out on Great Knott Field. The electrified and control areas were each of 1 acre, the control area lying about 110 yds. to the south-west of the electrified area.

Apparatus. This was the same as that used for the experiments carried out at Rothamsted in 1919. It supplied the clover and wheat as well as the barley plots.

Field Installation. This was also the same as in the experiment on this field with winter wheat in 1919. The wires were 10 ft. and 5 ft. apart respectively over the two halves, E. I and E. II, of the electrified plot.

Current. The discharge wires were kept at a voltage which varied between 20,000 and 50,000. The discharge current varied from 0·1 to 0·3 milliamp. per acre in the early part of the experiment, being rather low at first owing to the small power of the dynamo. After the conclusion of the clover experiment at the end of June the current varied generally from 0·8 milliamp. per acre.

Period of Discharge. The discharge was usually given from 7–11 a.m. and 1–5 p.m. (G.M.T.). It was started on April 9th and continued up to August 17th, the total period of the discharge being 786 hours.

Crop Results.

Electrified (Yield per acre)		Control (Yield per acre)	
E. I		**C. I**	
Grain ...	31·7 bushels	Grain ...	29·5 bushels
Straw ...	17·3 cwt.	Straw ...	16·0 cwt.
E. II		**C. II**	
Grain ...	33·0 bushels	Grain ...	25·17 bushels
Straw ...	18·5 cwt.	Straw ...	12·5 cwt.
Mean		**Mean**	
Grain ...	32·4 bushels	Grain ...	27·3 bushels
Straw ...	17·9 cwt.	Straw ...	14·3 cwt.

Difference between mean yield of electrified and control plots:

Grain +19 %   Straw +25 %

The increases in yield obtained are not very high, but they may be considered as definitely significant.

3. *Experiment with Winter Wheat (Yeoman).* This experiment was carried out on the same field as that used for the clover experiment.

The four plots were each 1/4th of an acre in extent and lay parallel with one another, the two electrified plots being contiguous, and the two control plots lying respectively north and south of the electrified ones and about 50 yards distant.

Apparatus. This was the same as used at Rothamsted Station in 1919; it also supplied current to the clover and barley experiments.

Field Installation. This was of the usual type, with cables about 7 ft. high and thin galvanised steel wires (s.w.g. 29) 10 ft. and 5 ft. apart respectively over the plots E. I and E. II.

Current. The voltage (crest value) varied between 20,000 and 50,000. The total discharge current was usually at the rate of 0·2 and 0·4 milliamp. per acre, but sometimes reached 0·8 in the later periods of the season.

Period of Discharge. The total period during which the discharge was given was 727 hours, the daily periods being usually 7–11 a.m. and 1–5 p.m. (G.M.T.). The discharge was given from April 9th to August 8th.

The grain was sown on September 25th, cut on August 9th, and carried on August 21st.

Crop Results.

Electrified (Yield per acre)		Control (Yield per acre)	
	E. I		C. I
Grain ...	18·84 bushels	Grain ...	20·4 bushels
Straw ...	19·0 cwt.	Straw ...	20·5 cwt.
	E. Il		C. II
Grain ...	18·35 bushels	Grain ...	18·24 bushels
Straw ...	19·7 cwt.	Straw ...	17·4 cwt.
	Mean		Mean
Grain ...	18·6 bushels	Grain ...	19·32 bushels
Straw ...	19·5 cwt.	Straw ...	18·9 cwt.

Difference between mean yield of electrified and control plots:

Grain −4 %   Straw +3 %

The differences are not significant. It is to be noted that the yield of all the plots is extremely low, being less than half of a normal good yield. This is presumably to be ascribed to the unfavourable season. It is also to be noted that as the crop was winter sown and the electrification was not started until April the crop was not subjected to the discharge during its early stages of growth.

Estimations of the nitrogen content of the surface soil of the various plots gave C. I, 0·15 per cent.; C. II, 0·15 per cent.; E. I, 0·16 per cent.; E. II, 0·15 per cent.

Experiments at Harper Adams Agricultural College.

1. *Experiment with Oats.* This experiment with oats (Svalof Crown) was carried out on the same field as the experiment of 1919, in which oats were also employed. The two electrified plots were contiguous, and each 1/3rd of an acre in extent.

Apparatus. The apparatus was exactly the same as that used in the similar experiment of 1919, viz., a motor-generator fitted with a Newton and Wright disc rectifier, and an oil-cooled transformer.

Field Installation. This was exactly similar to that employed in 1919, the fine wires being 10 ft. apart over plot E. I and 5 ft. apart over plot E. II. A screen of wire netting 8 ft. high was placed a few yards from the control, between it and the electrified plots. As the electrified plots were similarly screened by a belt of trees, such netting was not likely to give unfair protection to the control area.

Current. The voltage (crest value) varied from 20,000 to 60,000. The discharge current was kept at about 0·7 milliamp. which, if the current is proportional to the closeness of the wires, should give a current of about 0·5 and 1·6 milliamps. per acre over the plots E. I and E. II respectively.

Period of Discharge. Electrification was started on March 31st and continued to August 15th, the total number of hours during which the discharge was given being 793. The usual daily periods were from 7–11 a.m. and from 3–6 or 7 p.m. (G.M.T.).

Crop Results.

(Yield per acre)

	Electrified		Control	
	Grain	Straw	Grain	Straw
E. I	50·0 bushels (42 lbs.)	22·0 cwt.	56·0 bushels	24 cwt.
E. II	52·5 ,, ,,	22·75 ,,		
Mean ...	51·7 ,, ,,	22·4 ,,		

Difference between mean yield of electrified plots and control plots:

Grain − 8·5 %   Straw − 5·9 %

The results of the nitrogen estimations of the soil, which were carried out at the Rothamsted Experimental Station, are given below. The nitrogen content is slightly higher on the control area.

Percentage of N in dry matter

			Surface	Subsoil
C.	...	...	0·14	0·05
E. I	...	...	0·12	0·06
E. II	...	...	0·13	0·06

In considering the significance of the result it is to be noted that the yield from the control area is much below normal. The Principal of the Harper Adams College reports that in an average year a yield of from 75 to 90 bushels per acre is to be expected from this variety of oats. Some injurious factor, probably the wet season, must have been at work.

2. *Experiments with Clover-Hay.* This experiment was carried out on the same area as the oats experiment of 1919, "seeds" having been sown with the oats. The two electrified plots were each 3/4ths of an acre, and the control plot half an acre, in extent.

Apparatus and Installation. This was the same as in 1919, and the discharge was obtained from the same transformer as in the experiment with oats described above. The wires were 10 ft. apart over plot E. I and 5 ft. apart over plot E. II.

Current. The current supplied was rather high, as the voltage (crest value 20,000 to 60,000) was regulated to control the discharge over the oat plots. It varied from about 1·3 to 2·6 milliamps. per acre over E. I and from 0·7 to 1·3 milliamps. per acre over E. II, the higher range of currents occurring in the later stages of growth.

Period of Discharge. The discharge was given for 793 hours in all, being started on March 31st and continued to June 23rd. The daily periods were from 7–11 a.m. and from 3–6 or 7 p.m. (G.M.T.).

Crop Results.

	E. I	E. II	C.
Yield per acre (cwt.) ...	52·8	39·2	49·0
Difference between mean yield of electrified areas and control area			− 6 %

The results of the nitrogen estimations of the soil, made at the Rothamsted Experimental Station, are as follows:

Percentage of N in dry matter

				Surface	Subsoil
C.	...	...	...	0·13	0·06
E. I	...	...	...	0·12	0·05
E. II	...	...	...	0·13	0·05

In view of the large difference in crop yield between E. I and E. II the result is hardly significant.

GENERAL CONCLUSIONS FROM FIELD EXPERIMENTS.

A table is appended showing the results of the field experiments described in this paper. To this list is also added three earlier experiments with oats at Lincluden with which the author was associated, namely, the experiment of 1915 described by Mr Jørgensen(8) and that of 1916 described by the author and Mr Jørgensen(3), and that of 1917 an experiment similar to that of 1916 but hitherto unpublished. In the four early experiments of 1915–17 the discharge current from the wires was not measured.

From this list of experimental results the experiment of 1919 at Rothamsted with spring-sown wheat is excluded as the crop was a failure —the control plot yielding only eight bushels to the acre.

The difference in yield between the electrified and control plots both in bushels per acre and in percentages are given. In calculating the mean of these differences the results for the small barley plots of 1917 at Rothamsted have not been included as the crop was harvested before maturity.

Spring-sown Cereals.

				Difference between yield of electrified and control areas	
				Bushels per acre	%
Lincluden	...	1915	Oats	+ 4·8	+30
„		1916	„	+11·2	+49
„		1917	„	+ 0·7	+ 2
„		1918	„	+26·7	+50
„		1919	„	+12·8	+35
„		1920	„	− 2·6	− 6
„		1920	„	+18·8	+57
Rothamsted	...	1917	Barley, small plots	(+ 5·3)	(+36)
„		1918	„ „	+ 4·4	+10
„		1920	„ „	+ 5·1	+19
Harper Adams College		1919	Oats	+ 1·0	+ 2
„ „		1920	„	− 4·3	− 9
		Mean		+ 7·1	+22 %

Winter-sown Wheat.

Rothamsted	...	1919	—	+ 6·0	+38
„		1920	—	− 0·7	− 4

Clover-Hay.

Rothamsted	...	1919	1st crop	+11·7 cwt. per acre	+50
„	...	1919	2nd „	+ 4·3 „ „	+34
Harper Adams College		1920	—	+ 0·5 „ „	+ 2
„	„	1920	—	− 3·0 „ „	− 6
		Mean ...		+ 3·4 „ „	+20 %

The data show that of 18 field experiments—extending over six years—in which various crops were employed, 14 showed increased yields following electrification, while four showed decreased yields. Such a result is in itself sufficient to demonstrate the favourable effect of the electrical discharge; for a distribution of results of this kind could hardly be brought about by the chance superiority of the areas chosen for electrification. A closer examination of the comparative yields gives further support to this view, for of the positive results only three show increases of less than 10 per cent. and nine show increases of 30 per cent. and over, some reaching 50 per cent. and more; while of the four negative results none shows a decrease of as much as 10 per cent. The "scatter" of such results could not be the product of chance.

If the 12 experiments with spring-sown cereals are considered alone 10 are positive and 2 negative. Of the positive results only two are less than 10 per cent., while six show increases of 30 per cent. to 57 per cent.; on the other hand both the negative results are quite small, being 6 per cent. and 9 per cent. respectively. The effect of electrification in increasing the yield of spring-sown oats and barley has thus been demonstrated. The mean increase obtained was 22 per cent.

These results, together with the record of pot-culture experiments put forward in the second paper of this series (2) *and the laboratory studies published elsewhere* (2), *provide converging evidence—from the field, from pot cultures, and from the laboratory—of the effectiveness of minute electric discharges in the stimulation of the growth of plants.*

A beneficial effect of the discharge on clover-hay is probable, while that on winter-sown wheat is still uncertain.

DISCUSSION OF RESULTS.

When the poverty of our knowledge of the proper conditions under which the electric discharge should be applied is considered it is somewhat remarkable that spring-sown cereals should have shown such a large mean increase as 22 per cent. with an intensity of current and a period of discharge which were chosen more or less arbitrarily. There can be little doubt that the degree of increased growth and yield depends on such factors as the strength of the discharge, the daily period of the discharge, the seasonal period of the discharge, etc. Furthermore, the optimum electrical conditions will no doubt vary with different crops and with different climatic conditions[1]. A study of the optimal condi-

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[1] The erratic nature of some of the results is possibly due to the dependence of the electrical effect on certain climatic conditions which may or may not be present in a given season or during some critical period of a given season.

tions for electro-culture is therefore a complex one requiring numerous experiments under different conditions; for such a study pot-culture experiments are far more suitable than field experiments. This was early realised and a series of pot-culture experiments were carried out contemporaneously with the field experiments. The results are embodied in the paper(4) which follows this.

It is almost certain that the mean increased yield of 22 per cent. for spring-sown cereals described in the present paper in no way represents the maximum that may be obtained by the use of the electric discharge. As our knowledge of optimal conditions develops there is every reason to believe that still greater increases may be obtained. In support of this view one may point to the fact that pot-culture results (4) indicate that the stage of growth at which the discharge is given is of great importance.

Nature of the Effect.

As experimental work is still in progress it does not seem profitable to discuss at any length the difficult question of the physiological action of the very minute current which the plants receive. It has been shown by the author(2) in a recent paper that weak electrical currents $(0.5 \times 10^{-10}$ amp.) are able to increase the rate of growth of the coleoptile of the barley seedling, and that this increased rate of growth continues for some hours after the cessation of the current. A square foot of soil under oats may bear 70 tillers or more, so that with a discharge current of 0.5 milliamp. per acre—of which only about half reaches the crop below the wires—the current passing through each stalk (tiller) must be roughly 1×10^{-10} amp., which is of the same order as that found to stimulate the growth of the coleoptile. Such an increase in the rate of growth is likely to play a part in the production of the increased yield observed in field experiments and in the pot-culture experiments described in the succeeding paper(4).

In addition to this effect on the rate of growth there seems to be some general metabolic effect, for it has frequently been observed both in field and pot-culture experiments that the experimental plants have a darker green colour than the controls. There is evidence also(4, p. 16) that there may be some differential effect of the discharge, the grain yield being increased to a greater extent than the total dry weight.

There is no evidence that the stimulating effect of the current can be associated—as has sometimes been suggested—with any gaseous products, such as oxides of nitrogen, produced by the high tension discharge. There is no evidence that the soil under the wires is richer

in nitrogen than that of the control experiments. In any case the amount of combined nitrogen produced must be small and particularly so in the case of the pot-culture experiments where the voltage is comparatively low. Furthermore only a portion of such nitrogenous products would reach the soil of small experimental plots and of pot-cultures; the additional quantity of nitrogen thus rendered available must be very minute in comparison with that already present in the soil.

A certain additional supply of energy is available to the plants exposed to the discharge, but it is easy to demonstrate that the increased yields obtained cannot be accounted for on this basis, since the extra energy is far too small in amount. The maximum energy supplied is only at the rate of 50 watts (1 milliamp. at 50,000 volts) per acre for 6 hrs. a day for 5 or 6 months. A crop of barley according to Pütter[16] yields about 7×10^6 calories per m^2, so that the total additional energy supplied is less than 0·2 per cent. of the energy actually absorbed by the plant from sunlight. Only a fraction of this additional energy is available for the plant while the increased yield is of the order of 20 per cent.[1].

Clearly the effect produced is out of all proportion to the energy supplied; the physiological effect may thus be classed as a *stimulation*. The effect of the discharge is to make the plants physiologically more efficient, though, as has already been stated, the exact form which this increased efficiency takes is still obscure.

Economic Aspects of Electro-culture.

At the present stage of the development of the subject of electro-culture it is hardly profitable to discuss its economic aspects. It is unlikely that, for example, 22 per cent. is the limit of increase that may be obtained with spring-sown cereals. Pot-culture experiments now in progress indicate that bigger increases may be obtained with such crops even with a discharge period much less than that of the whole growing season. Any conclusions drawn at the present time as to the economics of the electro-culture of farm crops would be likely therefore to require considerable modification in the immediate future.

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[1] Of the current discharged from the wires of the experimental installations only about one-half reaches the crop below; also the energy from sunlight absorbed by the crop is greater than that indicated above since a considerable amount of energy absorbed is lost again in respiration. The additional energy available as a result of electrification must be less than 0·1 per cent. of the radiant energy of sunlight utilised.

SUMMARY.

Four years' additional experimental work on the application of a high tension discharge to the growth of field crops is described. Ten experiments with spring-sown oats and barley, two with winter-sown wheat, one with spring-sown wheat, and four with clover-hay have now been described.

The discharge was usually given at the rate of 0·5 to 1·0 milliamp. per acre from thin insulated wires stretched above the crop at a height of about 7 ft. and charged to a voltage of 40,000 to 80,000 (crest value). The discharge was usually given for 6 hours a day in two periods, 3 hours in the morning and 3 hours in the afternoon.

If the two experiments with spring-sown oats described in previous papers are included and the experiment of 1919 with spring-sown wheat (which yielded only 8 bushels to the acre) is excluded a series of 18 results is available spread over a period of 6 years. Of these 18 field experiments with various crops, 14 gave positive results in favour of the electrified plots, while 4 showed negative results, i.e. decreased yields compared with the controls. Of the 14 positive results only 3 show increases of less than 10 per cent. while 9 show increases of 30 per cent. and over, some reaching 50 per cent. and over. Of the 4 negative none shows a decrease of as much as 10 per cent.

Of the 12 experiments with spring-sown cereals 10 were positive and 2 negative. Of the positive results only 2 show increases of less than 10 per cent. while 6 show increases of 30 per cent. to 57 per cent.; on the other hand both the negative results are quite small, being 6 per cent. and 9 per cent. respectively.

The effect of electrification in increasing the yield of spring-grown oats and barley has thus been demonstrated. The mean increase in yield for such crops was 22 per cent.

A beneficial effect of the discharge on clover-hay is probable while the effect on winter-sown wheat is still uncertain.

Our knowledge of the proper conditions under which the discharge should be given is still so meagre that there is no reason to believe that the increased yields here described are the maximum obtainable as a result of electrification.

The mode of action of the current in producing increased growth and yield is still obscure. In several cases the electrified field crops showed a deeper green tint than that of the controls, and work already published has shown that in the case of the coleoptile (plumule sheath) of barley

minute electric currents are able to bring about an increase in the rate of growth.

The effect of the discharge is of the nature of a stimulus. The additional energy available from the current is too small to have any direct effect since it is only about 1/1000th, or less, of the energy which the plant obtains from sunlight.

There is no evidence that gaseous products of the discharge play any part in the stimulation of growth. The additional supply of nitrogen brought to the soil from oxides of nitrogen produced as a result of electrification must be exceedingly small.

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POT-CULTURE EXPERIMENTS WITH AN ELECTRIC DISCHARGE[1].

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(With Plate II.)

In the previous paper (this *Journal*, p. 240) which deals with the results of four years' field experiments in electro-culture, it has been demonstrated that with certain crops an increased yield can be obtained by means of the overhead electric discharge. The increased yields, however, though very definite were obtained under electrical conditions which were arbitrarily chosen and were almost certainly far removed from optimal. The necessity of studying the effect of the variation of such conditions was early recognised, but owing to the large probable error of field experiments (especially in cases where they cannot be arranged on the chequer-board system) each experiment, if a definite result is to be obtained, must be carried out for a number of years without any serious variation of the conditions. As in the present investigation there are a very large number of factors requiring study (such as strength of the discharge, duration of the discharge, daily period of the discharge, seasonal period of the discharge, variation of such conditions with different crops, etc.) it is clear that very long periods of time or very numerous annual experiments would be required to settle by field experiments the most appropriate conditions for different plants. In pot-culture experiments however, where the probable error can be reduced to 2 or 3 per cent. or even lower, significant results can be obtained in a single year, and a series of experiments under varying conditions can be carried out at the same time.

As the results of these considerations pot-culture experiments, running concurrently with the field experiments, have been carried out at the

Rothamsted Experimental Station from 1918 onwards. The object of these experiments was in part to demonstrate, in a shorter time than field experiments would allow, the effect of the discharge, and in part, as indicated above, to study the result of varying the conditions under which the discharge was given.

Experimental Technique.

In all the experiments networks charged to a high voltage (5000–16,000, crest value) were suspended over the plants by insulating supports. The networks consisted of a rectangular framework of narrow wood which carried two sets of parallel wires (galvanised steel, 0·35 mm. diam.) (Pl. II, Figs. 1 and 2) about 2 in. apart, the two sets being at right angles to one another. The networks could be raised or lowered, and so by increasing or decreasing the size of the air gap between the charged wires and the tops of the plants the strength of the current passing to them could be regulated. The pots were placed on insulating supports and the current passing through the plants was led off from silver plates or wires placed in the soil at the bottom of the pots to a microammeter reading to 0·01 microamp. The control pots were "earthed" by means of similar plates or wires. The currents actually passing to the plants were liable to vary with changes in atmospheric conditions, but they were brought back to the required intensity at frequent intervals during the day. When not otherwise stated, the discharging wires were made positive.

The discharge was given only in the day-time, and unless otherwise stated between the hours of 8 a.m. and 6 p.m. and for about 3 hrs. in the morning and 3 hrs. in the afternoon; it was usually stopped on the occurrence of rain. All the times given are Greenwich Mean Time.

In 1918 and 1919 the large glazed pots ordinarily used in agricultural work were employed, but starting with Exp. XII in 1920 they were replaced by large flower pots (holding 27 lbs. of soil) rendered impervious by treatment with molten paraffin.

The dry weights given are of the aerial parts only of the plant, except in the case of the single water-culture experiment (Exp. V) in which it is that of the whole plant.

Experiments of the Year 1918.

These experiments were designed to demonstrate (1) the effect of the discharge in accelerating growth, and (2) to obtain some information as to the effect of various intensities of current on the early stages of the growth of cereals.

High voltage current was obtained by the use of a mercury interrupter, an induction coil, and Lodge valves; thus the current was unidirectional but not of the sine-wave type. The three intensities of current given in the first set of experiments were calculated from the area of the pots as of the order of 0·5, 5·0 and 50·0 milliamps. per acre respectively.

<div align="center">

Series I.

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Experiment I.

Wheat (White Victor, 1916) in greenhouse. Sown June 3rd, electrification (8 a.m. to 12 noon and 1 to 4 p.m., G.M.T.), started June 5th; harvested July 7th. Each series with 4 pots, each containing 13–15 plants.

		Current per plant amp. $\times 10^{-9}$	Av. dry wt. of 4 plants (grm.)	Ratio of dry wts.
Control	...	—	0·73 ± 0·02	100 ± 2·7
E. I	...	0·3	0·72 ± 0·03	99 ± 4·3
E. II	...	3·0	0·71 ± 0·02	97 ± 2·7

The differences fall within the probable error.

These plants were badly attacked by mildew (*Erysiphe graminis*) which was worst on E. II.

Experiment II.

Maize (White Horsetooth) in greenhouse. Sown June 21st; electrification started June 26th; harvested August 2nd. Each series of 6 pots with 24–30 plants in all.

		Current per plant amp. $\times 10^{-9}$	Av. wt. per plant (grm.)	Ratio of dry wts.
Control	...	—	1·39 ± 0·05	100 ± 3·6
E. I	...	0·5	1·43 ± 0·05	103 ± 3·6
E. II	...	5·0	1·21 ± 0·05	87 ± 3·6
E. III	...	50·0	1·24 ± 0·05	89 ± 3·2

The important deduction to be made from the above table is that the very high discharge rate applied in E. II and E. III is definitely injurious.

Experiment III.

Barley (Biffen's "Pure Line," P. 8, 1917) in greenhouse. Each series consisted of 10 pots, and each pot bore 3 plants arranged so as to reduce root interference to a minimum. The pots were given equal quantities of water, the water content being kept up to about 15 per cent. The experiment ran from June 22nd to July 29th, electrification being given for 184 hours.

		Current per plant amp. $\times 10^{-9}$	Av. dry wt. of 3 plants (grm.)	Ratio of dry wts.
Control	...	—	2·30 ± 0·03	100 ± 1·3
E. I	...	2·3	2·26 ± 0·05	98 ± 2·2
E. II	...	13·0	2·12 ± 0·05	92 ± 2·1
E. III	...	120·0	1·98 ± 0·05	86 ± 2·1

The plants of all the four series were badly mildewed. The injurious effect of high intensities of current is clearly marked in E. III.

Experiment IV.

Barley in the open. Each series consisted of 9 pots, each pot having 5 plants. The experiment ran from June 20th to July 30th and the discharge was given for 241 hours, an average of 6 hours a day. Owing to an accident the "control" and part of one of the electrified sets were lost.

		Current per plant amp. × 10⁻⁹	Dry wt. of 45 plants (grm.)	Ratio of dry wts.
E. I	...	2·6	162·2 ± 2·2	100 ± 1·4
E. II	...	24·0	131·9 ± 5·5	81 ± 3·5
E. III	...	175·0	110·5 ± 4·0	68 ± 2·4

Some mildew was present on these plants. It was clearly noticeable that the plants most affected were those of E. III, the set subjected to the strongest current. The injurious effect of the strong current used in E. II and E. III is clearly brought out.

Experiment V.

Water Culture Experiment in greenhouse. Barley (Biffen's "Pure Line," P. 8, 1917); 15 plants in each series; July 5th to August 7th; 120 hours' electrification.

		Current per plant amp. × 10⁻⁹	Dry wt. per plant (grm.)	Ratio of dry wts.
Control	...	—	1·29 ± 0·08	100 ± 6·2
E. I	...	2·5	0·97 ± 0·03	75 ± 2·3
E. II	...	16·0	1·17 ± 0·02	90 ± 1·5
E. III	...	120·0	1·12 ± 0·03	87 ± 2·3

These plants were very badly attacked by mildew owing to damp weather conditions in July, the results are therefore untrustworthy.

Series II.

Since the earlier series of pot experiments had clearly shown (see especially Exps. II, IV and V) that higher currents were injurious, a new series was started in which the maximum current applied corresponded with the minimum of the first series. Owing to the lateness of the season only two experiments could be carried out.

Experiment VI.

Maize (Carter's White Horsetooth with grains graded between 0·5 and 0·55 grm.) was used in this experiment, which was carried out in the open. As before there were four series, each series consisting of ten pots bearing 3–5 plants each. The experiment lasted from August 19th to September 23rd, and the discharge was given for 135 hours.

Maize was selected for this and for Exps. II and VII, since the high temperatures of August made the growth of wheat and barley difficult. Maize also is immune from attack by mildew.

The current intensities used were at the rate of 0·5, 0·25, and 0·05 milliamp. per acre.

		Current per plant amp. × 10⁻⁹	Dry wt. of 30 plants (grm.)	Ratio of dry wts.
Control	...	—	7·78 ± 0·23	100 ± 3·0
E. I	...	0·3	8·12 ± 0·23	104 ± 2·9
E. II	...	1·4	8·37 ± 0·20	107 ± 2·6
E. III	...	3·8	7·41 ± 0·17	95 ± 2·2

The increases here in E. I and E. II are hardly significant since they are only about twice the probable error. Owing to the lateness of the season the plants suffered from night frosts, so that a limiting factor was very likely at work. The weight attained is markedly less than that of the greenhouse plants of the next experiment.

Experiment VII.

This experiment with maize was similar to that of Exp. VI, but was carried out in the greenhouse instead of in the open; each pot bore in nearly all cases three or two plants. The experiment lasted from August 23rd to September 25th, and the discharge was given during 118 hours.

		Current per plant amp. × 10⁻⁹	Dry wt. of 30 plants (grm.)	Ratio of dry wts.
Control	...	—	8·54 ± 0·40	100 ± 4·7
E. I	...	0·3	10·84 ± 0·28	127 ± 3·3
E. II	...	1·7	10·36 ± 0·24	121 ± 2·8
E. III	...	3·7	10·85 ± 0·50	127 ± 5·8

These results may be considered as definitely significant, since the increases observed are many times the probable error and they occur in all the electrified series. The results indicate that, in the case of maize at least, the discharge has an accelerating effect on the vegetative growth of plants under the conditions of pot-experiments. As the plants were only a month old the differences observed must be considered very marked. It is interesting to note that the weakest current seems as effective as the strongest.

Conclusions from Pot-culture Experiments of 1918.

The experiments with maize under greenhouse conditions have shown that, in the early stages of growth at least, currents of 0·3 to 3·7 × 10⁻⁹ amp. per plant have an accelerating effect on growth as shown by dry weight production, increases of 27 per cent. being obtained after only a month's growth. It has further been shown, as was to be expected,

that with higher current the stimulating effect passes over into one of retardation, currents of the order of 1×10^{-8} amp. an⒟ higher being injurious.

Experiments of the Year 1919.

The experiments of 1918 were designed to test the upper limits of useful discharge, *i.e.* the maximum current intensities which could be used without injury to the plant. The experiments of the season of 1919 were designed to confirm those of the year before and to obtain evidence as to the effect of much weaker currents. In 1918 the current intensity of about 0.3×10^{-9} amp. per plant was used and also currents of 10 times and 50 times this intensity. In 1919, taking 3.0×10^{-9} amp. as the unit, currents of 1/10th and 1/50th of this were also given. The discharge was usually given from 7–10 a.m. and 3–6 p.m. The plants were grown in the usual glazed pots to the soil of which 10 per cent. sand was added and also a dressing of 1 per cent. chalk, 0.1 per cent. nitrate of soda, 0.1 superphosphate, and 0.05 per cent. potassium sulphate.

Experiment VIII.

Maize (Sutton's Giant Horsetooth) grown in greenhouse. The seeds were graded between 0.40 and 0.45 grm.; sown June 14th; electrification started June 22nd; total hours of discharge 206; harvested August 1st. The control series and E. I each consisted of 9 glazed pots, E. II of 28 pots; each pot had 3 plants.

	Current per plant amp. $\times 10^{-9}$	Dry wt. per pot (grm.)	Ratio of dry wts.
Control I ...	—	5.62 ± 0.14	100 ± 2.3
Control II ...	—	5.67 ± 0.12	
E. I ...	3.0	5.70 ± 0.15	100 ± 2.6
E. II ...	0.12	6.81 ± 0.09	120 ± 1.6

The 20 per cent. difference shown by E. II is definitely significant since it is over seven times the probable error of the difference. It confirms the 27 per cent. increase obtained in 1918 with maize under greenhouse conditions. In that year the plants given a current of 3×10^{-9} amp. per plant also gave an increase.

The nitrate in the soil of the pots was determined at the end of the experiment with the following result:

C. I ...	53.50 pts. per million	
C. II ...	52.97 ,,	,,
E. I ...	54.68 ,,	,,
E. II ...	43.83 ,,	,,

The increased yield in E. II was not associated with an extra supply of nitrates in the soil.

Experiment IX.

Maize in the greenhouse. This experiment was a repetition of Exp. VIII above. The control and electrified sets each consisted of 28 pots; each pot had 3 plants; no manure was given. Planted August 26th; discharge started September 5th; harvested September 29th; hours of discharge, 134.

	Current per plant amp. $\times 10^{-9}$	Dry wt. per pot (grm.)	Ratio
Control ...	—	$2 \cdot 32 \pm 0 \cdot 055$	$100 \pm 2 \cdot 4$
E.	$0 \cdot 12$	$2 \cdot 52 \pm 0 \cdot 038$	$109 \pm 1 \cdot 6$

The plants were only growing for a month, so that a large difference could hardly be expected. The difference is between three and four times the probable error and may be taken as significant.

Experiment X.

Maize in the open. Control, 23 pots; E. I, 12 pots; E. II, 12 pots; E. III, 56 pots; 3 plants in each pot. Grain planted 10th June, above ground June 17th; discharge given from June 18th to September 12th; total hours of discharge 359. Plants harvested September 13th.

The plants came up very evenly, but soon after they were up a cold spell gave them a severe check and some were killed.

	Current per plant amp. $\times 10^{-9}$	Dry wt. per pot (grm.)	Ratio
Control ...	—	$17 \cdot 28 \pm 0 \cdot 18$	$100 \pm 1 \cdot 0$
E. I ...	$3 \cdot 0$	$16 \cdot 73 \pm 0 \cdot 22$	$97 \pm 1 \cdot 3$
E. II ...	$0 \cdot 31$	$16 \cdot 03 \pm 0 \cdot 16$	$93 \pm 0 \cdot 9$
E. III ...	$0 \cdot 06$	$15 \cdot 01 \pm 0 \cdot 29$	$92 \pm 1 \cdot 7$

The superiority of the control pots is probably to be explained by the presence of a wire screen between them and the electrified pots. This screen was arranged to guard the control plants from stray currents, but unfortunately it also gave a certain amount of shelter and protected the plants from north winds. The low yield of E. III is probably to be explained by the crowding of the 56 pots which was necessary to obtain the lowest possible current per plant.

Experiment XI.

Barley (Biffen's Archer Y. 83) in the open. Grain graded between 0·04 and 0·05 grm.; sown May 22nd in large glazed pots containing 32 lbs. of soil; 3 plants in each pot, discharge ran from June 5th to September 12th; harvested September 24th. The plants received a heavy dressing of nitrate of soda, as a result of which they were severely attacked by mildew and rust, became badly lodged towards the end of August, and were also very late in ripening.

		Current per plant amp. $\times 10^{-9}$	Grain per pot (grm.)	Ratio	Total dry wt. per pot (grm.)	Ratio
Control I	...	—	18·69	100	64·37 ± 1·23	100 ± 1·9
E. I ...	...	3·0	13·72	73	60·51 ± 1·24	94 ± 1·9
E. II ...	...	0·33	15·84	85	63·68 ± 0·91	99 ± 1·4
Control II	...	—	16·89	100	63·93 ± 0·76	100 ± 1·2
E. III ...	...	0·06	11·75	70	56·74 ± 0·90	89 ± 1·4

The results are very erratic, which must be put down to the attack of mildew and to the effect of bad weather in causing lodging of part of the crop. The superiority of the controls is probably due to protection by the wire screen. Control II was not lodged at all and Control I less than the electrified sets; thus the results of Exp. XI are clearly untrustworthy.

Conclusion from Pot-culture Experiments of 1919.

The results of 1919 have confirmed those of 1918, for both experiments with maize under greenhouse conditions gave a definite increase of dry weight as a result of stimulation by the discharge, even with a current as low as 1×10^{-10} amp. per plant. Experiments carried out in the open with Barley and Maize showed no effect from the current, but the conditions were not satisfactory.

EXPERIMENTS OF THE YEAR 1920.

The pot-culture experiments of 1920 were conducted in the same way as those of 1919, and the discharge was given for the same periods. In Exp. XII the usual glazed pots were used, but for Exps. XIII and XIV the plants were grown in large flower pots which had been rendered non-porous by treatment with molten paraffin. In these pots the drainage hole was left at the bottom and the necessary water was supplied through a small flower pot sunk in the soil of the large pot. In this season's experiments only medium and weak currents were employed, but the effect of alternating current was also investigated.

In 1918 and 1919 the high tension discharge was obtained by the use of a mercury interrupter and an induction coil, Lodge valves being employed for rectification. In the experiments of 1920 the installation consisted of a small rotary converter (giving 70 volts A.C. with a frequency of 50 per sec.) and a wax-impregnated transformer; the current was thus of the sine-wave type. The overhead networks supplying alternating current were connected directly to the transformer; for those giving direct current rectification was obtained by means of Lodge valves. The alternating currents employed were too small to be measured, but the networks supplying these were kept at the same heights as those

supplying direct current. The voltage employed was from 4,000 to 16,000 (crest value), varying with the growth of the plants and with weather conditions.

The plants themselves were able to bring about some slight rectification, for a moving coil ammeter in the alternating current circuit showed a just observable deflection when the networks were a little lower than those over plants which were receiving weak, direct current.

Experiment XII.

Maize (Sutton's Giant Caragua) grown in greenhouse; grain graded between 0·37 and 0·47 grm.; sown May 22nd; electrification started May 29th; total hours of discharge 321; harvested August 4th. The control set and E. I each consisted of 10 pots, and E. II of 9 pots; each pot bore 3 plants. The pots held 7 lbs. of soil, and to each was added 2 grms. superphosphate, 2 grms. sodium nitrate, and 1 grm. potassium sulphate.

	Current per plant amp. × 10⁻⁹	Dry wt. per pot (grm.)	Ratio of dry wts.
Control ...	—	$14·52 \pm 0·17$	$100 \pm 1·2$
E. I (D.C.) ...	0·12	$15·66 \pm 0·11$	$108 \pm 0·8$
E. II (A.C.) ...	?	$15·39 \pm 0·18$	$106 \pm 1·2$

Both the direct and alternating current produced a definite increase of yield (Pl. II, Fig. 3), though the effect with direct current is somewhat less than that obtained with maize in 1918 and 1919.

The total nitrogen and the nitrate in the soil of these pots were determined with the results shown below.

			Percentage of N in dry soil	Nitrate parts per million
Control	...	...	0·128	11·59
E. I ...	...	...	0·123	7·53
E. II	...	...	0·124	7·69

The increased yield of the electrified crops is clearly not associated with an extra supply of nitrates or of nitrogen in the soil.

Experiment XIII.

Barley (Biffen's "Pure Line" 47/51/) grown in the open; grain graded between 0·05 and 0·06 grm.; sown in large pots containing 27 lbs. soil. The discharge ran for 351 hours, from May 12th to July 26th; harvested July 27th. The control, E. I, and E. III consisted each of 9 pots, E. II of 18 pots. Each pot had 5 plants. The fertiliser consisted of 2 grms. superphosphate and 0·5 grm. sodium nitrate per pot.

	Current per plant amp. × 10^{-9}	Dry wt. per pot (grm.)	Ratio of dry wts.
Control ...	—	23·88 ± 0·79	100 ± 3·3
E. I (D.C.) ...	1·05	23·84 ± 0·32	100 ± 1·3
E. II (D.C.) ...	0·05	23·28 ± 0·33	98 ± 1·4
E. III (A.C.)...	?	26·33 ± 0·36	110 ± 1·3

The direct current has produced no effect, but the alternating current shows an increase which is several times the probable error and is probably significant.

Experiment XIV.

Wheat (Little Joss) in the open; grain graded between 0·055 and 0·065 grm.; sown May 5th in large pots containing 27 lbs. of soil; discharge ran for 391 hours from May 17th to August 12th; harvested August 13th. The control, E. I and E. III consisted each of 9 pots, and E. II of 18 pots; each pot had 5 plants. The fertiliser consisted of 2 grms. superphosphate, 2 grms. sodium nitrate and 1 grm. potassium sulphate per pot.

	Current per plant amp. × 10^{-9}	Dry wt. per pot (grm.)	Ratio of dry wts.
Control ...	—	16·22 ± 0·14	100 ± 0·8
E. I (D.C.) ...	3·0	17·66 ± 0·38	105 ± 2·3
E. II (D.C.) ...	0·1	17·11 ± 0·16	102 ± 0·9
E. III (A.C.)...	?	18·76 ± 0·36	112 ± 0·9

The effect of the direct current is doubtful, but the alternating current has given an increase of yield which is nearly six times the probable error and is therefore significant.

Conclusions from Pot-culture Experiments of 1920.

The experiments of this year with maize have confirmed the result obtained in 1918 and 1919, that by means of direct current the yield in dry weight of the plant can be increased. The increase however is smaller than those of previous years; this difference may possibly be related to soil differences, that used in the experiments of 1919 being much richer in nitrogen. There is also the possibility that the difference may be due to the difference in the type of discharge employed this year as compared with that of previous years.

Experiments undertaken with the discharging networks supplying very weak alternating current show that such a system is as effective or possibly more effective than one supplied with direct current, for by its means increases have been obtained with both barley and wheat. Whether, however, the effect can be ascribed to an alternating current passing through the plant or to the "rectified" current which the plant itself causes is still uncertain.

EXPERIMENTS OF THE YEAR 1921.

The pot-culture experiments of 1921 were conducted in the same way as those of 1920. Large flower pots, which had been rendered impervious by treatment with molten paraffin, were used for all the experiments; each pot held about 27 lbs. of soil which had been mixed with 10 per cent. of sand.

The experiments of 1921 were designed: (1) to continue the tests made in 1920 as to the relative effects of direct and alternating current; (2) to compare the effects of an "upward" and a "downward" current through the plant; (3) to obtain some idea as to the stage of the plant's growth at which the discharge is most effective. The discharge networks supplying alternating current were kept as in 1920 at the same height as those supplying direct current. Owing to their very small intensity the actual strength of the alternating currents was not measured.

Experiment XV.

Barley (Goldthorpe, "Pure Line"); grain graded between 0·06 and 0·05 grm.; sown April 26th; electrification from May 11th to June 12th; total time of discharge 173 hrs.; harvested June 13th. The current was increased during the discharge period by three increments from 0·028 to 0·16 × 10⁻⁹ amp. Each pot held 5 plants, and 1·0 gm. super-phosphate, 0·5 gm. sulphate of potash and 0·5 gm. nitrate of soda per plant was given. The results were as follows:

	Current per plant amp. × 10⁻⁹	Dry wt. per plot (grm.)	Ratio of dry wts.
Control ...	—	10·29 ± 0·12	100 ± 1·2
E. I (D.C.) ...	0·028—0·16	10·60 ± 0·15	103 ± 1·5
E. II (A.C.) ...	?	♦1·79 ± 0·30	115 ± 2·9

The plants subjected to the alternating current show an increase of 15 per cent. which is about five times the probable error and is therefore significant. The increase of E. I is too small to be significant.

Experiment XVI.

This experiment was the same as Exp. XV except that the electrification was continued until July 5th, when the plants were harvested; total time of discharge, 274 hours. Each set consisted of 6 pots, but only the plants of three pots of E. I could be harvested as the others were damaged by corn-fly. The current was increased during the discharge period by five increments from 0·028 to 0·33 × 10⁻⁹ amp. The results were as follows:

	Current per plant amp. × 10^{-9}	Dry wt. per pot (grm.)	Ratio of dry wts
Control ...	—	$31 \cdot 5 \pm 0 \cdot 4$	$100 \pm 1 \cdot 3$
E. I (D.C.) ...	0·028—0·33	$34 \cdot 0 \pm 0 \cdot 13$	$108 \pm 0 \cdot 41$
E. II (A.C.) ...	?	$37 \cdot 2 \pm 0 \cdot 62$	$118 \pm 2 \cdot 0$

Both electrified sets show significant differences, that subjected to alternating current showing the greater.

Experiment XVII.

In this experiment the control sets and E. I and E. II were exactly similar to those of Exps. XV and XVI above, but electrification was continued until July 15th, the total time of discharge being 363 hours. The current was increased during the discharge period by seven increments from 0·028 to 0·44 × 10^{-9} amp. E. III and E. IV consisted of pots which had been treated similarly to those of E. I and E. II respectively of Exp. XV, but had since that time (June 12th) received no electrification, *i.e.* they had received the discharge only for the first month of their growing period. The plants were harvested on August 11th, when they were fully ripe.

Control II and E. V consisted of pots which were treated similarly to Control I and E. I respectively, but they were lightly manured, receiving only half the amount of potash and of superphosphate supplied to the other sets, and no nitrogen. The results were as follows:

	Current per plant amp. × 10^{-9}	Dry wt. per pot (grm.)	Ratio of dry wts.	Grain per pot (grm.)	Ratio of grain yield
Control I	—	$46 \cdot 5 \pm 0 \cdot 8$	$100 \pm 1 \cdot 7$	9·57	100
E. I (D.C.). Full period ...	0·028—0·44	$49 \cdot 2 \pm 0 \cdot 68$	$106 \pm 1 \cdot 5$	8·90	93
E. II (A.C.). ,, ,, ...	?	$53 \cdot 0 \pm 0 \cdot 70$	$114 \pm 1 \cdot 5$	11·14	116
E. III (D.C.). 1st month	0·028—0·16	$48 \cdot 5 \pm 1 \cdot 16$	$104 \pm 2 \cdot 5$	12·1	126
E. IV (A.C.). ,, ,,	?	$51 \cdot 9 \pm 0 \cdot 86$	$112 \pm 1 \cdot 8$	11·5	120
Control II	—	$21 \cdot 2 \pm 0 \cdot 57$	$100 \pm 2 \cdot 7$	4·70	100
E. V (D.C.). Full period	0·028—0·44	$22 \cdot 8 \pm 0 \cdot 26$	$108 \pm 1 \cdot 2$	5·70	121

Of the plants (E. I and E. II) subjected to the discharge during the whole of their period of active growth, both sets show a definite increase in dry weight, the effect from alternating current being the larger. The yields of E. III and E. IV show, however, that the increase in dry weight is of the same order when the plants are subjected to the discharge for the first month only of their growing season, indicating that such short treatment is as effective as one applied throughout the growing season. This deduction is supported by evidence from the yields of grain, where the short period of electrification seems even more effective than the longer; the data of grain yields are subject, however, to such a large experimental error (which unfortunately was not determined), that without confirmation they must be received with caution.

The growth of the lightly manured plants (Control II and E. V) has been reduced by the poor supply of mineral salts to less than half that of the others; the discharge, however, has had a definitely stimulating effect.

Experiment XVIII.

This experiment with barley was designed to test the effect of the normal atmospheric current. Nine pots were subjected to the same conditions as the control plants of Exp. XVII above, but they were placed in a "cage" consisting of horizontal "earthed" wires, about $2\frac{3}{4}$ ins. apart, fixed to four wooden supports. The wires were so thin (0·35 mm. diam.) that they could not appreciably modify the light intensity, air movement or rainfall to which the plants were subjected. The results were as follows:

	Dry wt. per pot (grm.)	Ratio of dry wts.
Control ...	46·5±0·8	100 ±1·7
"Caged" ...	44·3±0·72	95·3±1·6

The difference between the two yields is 4·7 per cent., the probable error of which is ± 2·3; the difference is thus too small to be definitely significant. It indicates, however, that the effect of the earth's normal field, if favourable at all, is only slight. This experiment is being continued.

Experiment XIX.

Maize (Sutton's Giant Horsetooth) in greenhouse; grain graded between 0·4 and 0·5 grm. sown May 6th, electrification started May 18th; total time of discharge, 192 hours; harvested June 21st; the current was increased during the discharge period by two regular increments from 0·13 to 0·27 × 10⁻⁹ amp. Each pot had five plants, and to each pot 5 grms. superphosphate, 5 grms. nitrate of soda and 2·5 grms. sulphate of potash were added. Each set consisted of 15 pots. The results were as follows:

	Current per plant amp. × 10⁻⁹	Dry wt. per pot (grm.)	Ratio of dry wts.
Control ...	—	15·6 ±0·39	100 ±2·5
E. I (D.C.) ...	0·13—0·27	15·5 ±0·32	99±2·0
E. II (A.C.) ...	?	14·27±0·34	92±2·2

Both the direct current and the alternating current sets show somewhat less yields than the controls. This is the first of the experiments with maize under glass which has failed to show any significant increase.

Experiment XX.

Maize (Sutton's Giant Horsetooth) in greenhouse; grains graded between 0·45 and 0·35 grm.; sown June 25th; electrification started July 6th, total time of discharge 168 hours; harvested August 3rd. The current was started at $0·22 \times 10^{-9}$ amp., and increased about half-way through the experiment to $0·44 \times 10^{-9}$ amp. per plant. Each pot had three plants, with 3 grms. superphosphate, 3 grms. nitrate of soda and 1·5 grms. sulphate of potash. In E. I the discharge network was made positive and in E. II negative, so that the direction of the current was (conventionally) downward and upward respectively. The results were as follows:

	Current per plant amp. × 10⁻⁹	Dry wt. per pot (grm.)	Ratio of dry wts.
Control ...	—	16·60±0·39	100±2·3
E. I (wires +)	0·22—0·44	18·20±0·49	110±2·9
E. II (wires –)	,,	18·95±0·40	114±2·4

Both the sets subjected to the discharge show an increase, but the difference between the effect of the positive and negative discharge is too small to allow of any deduction as to their relative efficiency.

Conclusions from Pot-Culture Experiments of 1921.

Definite increases in growth were obtained in four out of five pot-culture experiments in 1921. The season's work has confirmed the results of 1920 that discharging a network supplied with alternating current is usually as effective, or even more effective, than direct current. The results obtained also point to the fact than an "upward" current through the plant can increase growth in the same way as the "downward" current hitherto used, though further experiments are required to determine which is the more effective. Evidence has also been obtained which indicates that a discharge applied for the first month only of the growing season may be as effective as one continued throughout the growing season.

The experiment in which barley plants were removed from the action of the normal atmospheric current shows that the favourable effect, if any, of the earth's electric field can only be slight.

GENERAL CONCLUSIONS.

The results of pot-culture experiments carried out with cereals during the years 1918–21 have shown the value of the method of experimentation both for demonstrating the stimulating effect of an overhead electric discharge on the growth of plants and also for a study of the best conditions (at present almost unknown) under which the discharge should

be given. In a series of experiments carried out with Maize, Wheat and Barley (both under glass and in the open) significant increases in dry weight have been obtained in a number of cases. Rejecting Exps. I, III, V, and XI, on the ground of attack by mildew, and Exp. IV because of the loss of the control set, and Exps. VI and X because of the effect of cold weather upon the Maize plants, and excluding also those experiments in which currents of a high and damaging intensity were used and there was a marked reduction of yield, there are left 28 experimental sets. Of these 28 sets 23 show an increased yield as compared with their respective controls, while five give a negative result[1]. Of these 23 positive results 14 exhibit differences which are at least three times the probable error, and in 11 these differences are at least four times the probable error. Of the 11 sets of Maize plants grown under glass 7 showed increased yields, in 6 of these the difference being at least three times and in 4 at least four times the probable error. The largest percentage increase in dry weight was $27 \pm 5 \cdot 7$, which was shown by Maize plants under glass and little more than a month old (Exp. VII); with Barley the largest dry-weight increase was $18 \pm 2 \cdot 4$ per cent. The average increase of yield was $11 \cdot 4$ per cent., but as the plants grown were of various kinds and various ages this number has not much significance.

Increased growth was obtained both with direct and alternating discharge, the second being apparently as effective, or possibly even more effective, than the direct current. As however the plants are able to bring about some rectification of the current it is not certain that the stimulating effect observed was actually due to such currents. Owing to their low intensity the alternating currents were not measured.

One of the most striking results of this work is the marked sensitiveness of the plant to currents of very low intensity; definite increases of dry weight were obtained with currents as low as 1×10^{-10} amp. per plant (Exp. IX), and laboratory studies[2] have shown that plants will respond to currents of even lower intensity. There is at present no information as to the minimum current to which the plant will respond. It may be, however, that the minimum is of the order of the earth's normal vertical current (1×10^{-16} amp. per cm.[2]), for Exp. XVIII leaves open the view that the earth's electric field may have a slight influence on the plant's growth[2]. Information is also lacking as to the optimum

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[1] If those rejected as a result of injury from mildew, frost, etc., are included, twenty-five sets out of forty-one show an increase.

[2] Since many plants grew satisfactorily in greenhouses and in the neighbourhood of trees where they are screened from the earth's field and deprived of the normal air-earth current the effect of this current can hardly be great.

intensity of current, but under the conditions employed it certainly appears to lie below 1×10^{-3} amp. per plant, as with currents of this order or a little higher decreased yields are obtained showing that the maximum has been passed. With currents of 0·1 microamp. a marked reduction of yield is obtained (Exps. II–V). The optimum and maximum and minimum currents are almost certain to vary with the type of discharge and the period during which it is employed, also with the kind of plant and the stage of growth of the plant, and with external conditions. The importance of the stage of growth of the plant at which electrification is given is well brought out in Exp. XVII where the increased dry weight was practically as great when the discharge was applied for the first month only as for an application during the whole growing season.

The results of Exp. XX indicate that an "upward" current (*i.e.* with the wires negatively charged) can stimulate the growth of Maize in the same way as the "downward" current generally used in these experiments. The observed difference between the two types of current is, however, too slight to decide their relative efficiency[1].

Most of the experiments described were concerned with the increase of dry weight and the sets were not carried to maturity; in Exp. XVII however both total dry weight and grain yield were determined, though unfortunately the grain from each pot was not weighed separately, so the probable errors could not be calculated. It is noticeable however that in four out of the five sets the percentage increase in grain yield is greater than the increase in total dry weight. In the absence of any knowledge of the probable errors of the grain yields any conclusion can only be tentative, but there is some indication that the electric current has a differential effect, reproductive growth as measured by grain yield being stimulated to a greater extent than vegetative growth as measured by total dry weight.

The record of field experiments put forward in the first paper(1), together with the results described here, and the laboratory studies published elsewhere (*loc. cit.*), *provide converging evidence, from the field, from pot-cultures and from the laboratory, of the effectiveness of minute electric discharges in stimulating the growth of plants.*

As experimental work is being continued it is not considered profitable in the present state of our knowledge to discuss the nature of the stimulation which the electric discharge effects. The laboratory studies

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[1] S. Lemström, as a result of a few imperfect experiments in which dry weights are given in only one case, and neither voltage, current nor experimental error was determined, put forward the view that the downward current is more effective than the upward one.

, cited above indicate that it is the weak current passing through th plant which is mainly or solely responsible for the increase in growth observed. The gross mechanical view put forward by Lemström that a positive current from the wires to the plant drives nutritive substances into the plant, while a negative current from the plant to the wires draws water and dissolved substances up the plant is of course hardly tenable.

It has been pointed out in the previous paper (p. 264) that the additional plant material produced is out of all proportion to any additional energy which the plant may gain directly from the discharge; the effect is accordingly to be classed as of the nature of a stimulation. The disproportion between cause and effect is well brought out in Exp. VII of this paper where 30 plants of Maize produced an additional dry weight of 2·3 grms. as a result of 118 hours' electrification. With a current of $0·3 \times 10^{-9}$ amp. per plant, and assuming a voltage of 16,000 throughout (which is certainly too high a reckoning), *the additional energy supplied is only about* 15 *calories* and not all of this is available to the plant; *the calorific value of the extra dry weight produced was however of the order of* 9000 *calories*. Also it is noticeable that there is no relation between the intensity of the current and the amount of additional plant material produced; higher currents do not bring about larger yields.

SUMMARY.

Pot-culture experiments carried out during a period of four years with Wheat, Barley and Maize show that these plants exhibit increase of dry weight when subjected to minute electric currents (of an intensity as low as $0·1 \times 10^{-10}$ amp. per plant) from wire networks charged to a high voltage suspended above them. Of 28 sets in which the cultural conditions were satisfactory 23 showed an increased yield.

A percentage increase in dry weight of $27 \pm 5·8$ was shown by Maize plants grown under glass and little more than a month old. With Barley the largest percentage increase was $18 \pm 2·4$.

Direct currents were mostly studied, but increased growth was obtained with both direct and alternating current, the alternating current being apparently as effective or even more effective than the direct; the plants themselves, however, are able to bring about a slight rectification of the current.

Electrification of Barley for the first month of the growing season

appears to be as effective as electrification during the whole growing season.

The discharging networks were usually charged *positively*, but a similar stimulating effect on dry weight production was obtained with a *negative* charge on the network.

Currents of the order of 1×10^{-8} amp. per plant and higher are injurious, causing a reduction of dry weight.

In one experiment plants screened from the normal atmospheric current by a series of parallel "earthed" wires (0·35 mm. diameter) showed, by comparison with the controls, a percentage *decrease* of $4·7 \pm 2·3$, which is hardly significant. It indicates however that though a favourable effect may be exerted by the normal atmospheric (air-earth) current, yet the effect can be but slight.

In one experiment with Barley indications of a differential effect of the discharge were obtained, for the increase in grain yield brought about by the discharge was greater than the increase in total dry weight.

The effect is to be classed as of the nature of a stimulation since the energy-value of the additional plant material produced is out of all proportion to the energy supplied by the electric discharge.

These experiments with pot-cultures fall into line with those from the field[1] and from the laboratory[2]; they leave no uncertainty as to the favourable action of the electric discharge.

LITERATURE CITED.

(1) BLACKMAN, V. H. Field Experiments in Electro-culture. *Journ. Agric. Sci.* **14**, 240–67, 1924.

(2) —— and LEGG, A. T., and GREGORY, F. G. The Effect of an Electric Current of Very Low Intensity on the Rate of Growth of the Coleoptile of Barley. *Proc. Roy. Soc.* B. **95**, 214–28, 1923.

(3) LEMSTRÖM, S. *Electricity in Agriculture and Horticulture,* London, 1904.

EXPLANATION OF PLATE II

Fig. 1. Photograph of a portion of the pot-culture installation of 1920. The pots on their insulated stands and the rectangular frameworks bearing the discharge networks are shown.

Fig. 2. A single experimental set of nine pots bearing Barley plants. Above is seen the framework of wood carrying the discharge wires which are too thin to be clearly visible; the mechanism by which the framework can be raised and lowered is to be seen. The insulated stand supports the pots, and on the right is seen the lead which carries to the microammeter the current passing through the pots.

Fig. 3. Maize plants from Exp. XII. The pot in the middle is from the unelectrified control set; that on the right from the set receiving a direct current of $0 \cdot 12 \times 10^{-9}$ amp. per plant; that on the left from the set receiving alternating current.
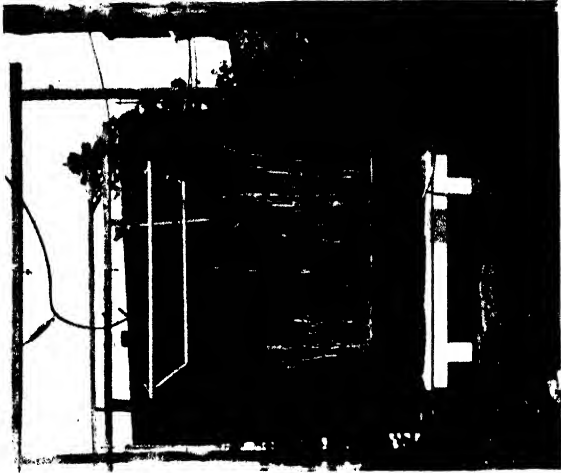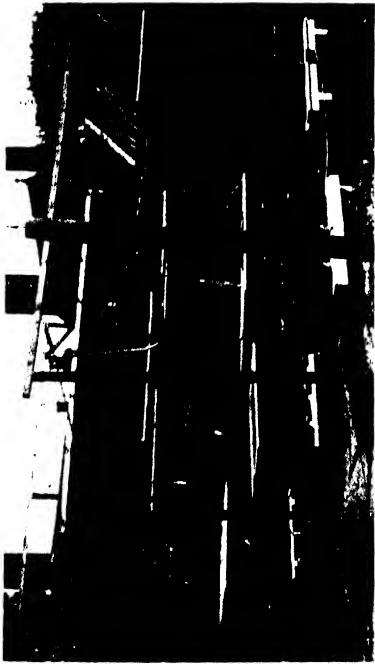
(Received November 23rd, 1923.)

Fig. 2

Fig

Fig. 3

INVESTIGATIONS ON YIELD IN THE CEREALS[1]. I.

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(With Three Text-figures.)

CONTENTS.

PART II (*continued*).

§ X. WEEKLY OBSERVATIONS ON DRY WEIGHT, NITROGEN CONTENT AND ASH CONTENT.

Final yield of grain and straw reflect, or are functions of, the combination of processes conveniently designated "growth." A successful analysis of "growth" would afford an analysis of "yield" and it is quite patent that "yield" must continue to be imperfectly understood so long as growth remains unanalysed. But although the full solution of the "yield problem" thus rests with plant physiology, it seems within the bounds of possibility that comparative studies upon varieties may bring to light distinctive (varietal) features of "growth" which are correlated with distinctive (varietal) features of "yielding power." One or more of these may possibly serve as an "index" of yielding power, as the touchstone which is so urgently needed in plant breeding. The reliability of comparative studies of this kind rests fundamentally upon accuracy

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[1] Part I and Part II, §§ I–VI with Appendices I, II and IV appeared in this *Journal*, Vol. XIII, Part 4, October, 1923. Part II, §§ VII, VIII and IX with Appendices III and V appeared in Vol. XIV, Part I, January, 1924. The paper will be completed in the next number of this *Journal*. In every number a complete bibliography is given but of the tables only those concerned are published.

of "sampling." For all the experimental attributes of freely tillering cereals, sampling is extremely difficult and the difficulties are most acute for "weight" attributes. Clearly then, a test of accuracy of sampling at all stages of growth is an indispensable preliminary. It was this consideration that determined the lines of the investigation to be recorded here and it is in the light of it that the results will be discussed.

For such preliminary work, average dry weight per plant, though in itself of no physiological interest, is the simplest observation: accordingly it was the principal one made.

Barley plants absorb from the soil varying amounts of ash (including SiO_2) per unit of dry weight formed. Differences of variety and of SiO_2 and water, etc., content of the soil determine these amounts and for comparative purposes dry weight excluding ash is a more useful variable than gross dry weight. For this, among other reasons, ash content determinations were made as far as possible.

In field crops of cereals an unhealthy condition commonly attributed to nitrogen-starvation is often in evidence and it frequently displays a differential incidence among varieties. This fact, coupled with certain physiological considerations which are discussed later on, made it desirable to determine nitrogen content in every sample lifted for dry weight.

Except for the early nitrogen and ash determinations, all observations were on the single-plant basis as on this basis alone can the reliability of small samples be tested.

(A) *Procedure.*

The plan of sowing has already been carefully described in § I. For dry weight, nitrogen, and ash determinations, the plants of Bed 8 were used (intervals = 2 inches from plant to plant and 1 foot from row to row).

The weekly sample of plants was obtained by lifting one, or if necessary to give a sufficient number, two of the rows. All plants which had failed to satisfy the requirements of uniformity [*vide* § IX above, conditions (i)–(iv)] were discarded. After the numbers of leaves and tillers had been counted, the plants were dried and the dry weight of every individual plant was found. In the early stages the nitrogen content of the sample as a whole was determined, but, as the plants grew larger, nitrogen and ash determinations were made on sub-samples of 3–5 plants and finally it was possible to estimate both the nitrogen and the ash of single plants.

At this point consideration must be given to the method of sampling employed. In work of this kind several methods have been tried. Perhaps the most generally adopted has been the selection from the complete experimental population of a specified number of plants which appeared to the eye to be representative of the average stage of development obtained by the population as a whole. For plants which can be grown at wide intervals, this rational method is quite practicable since the removal of a plant does not seriously affect the environment of its neighbours. Moreover it is possible to survey the whole population plant by plant and so to decide what is the average stage of development and to select plants representative of that stage. The case is different with a freely tillering cereal race, for sowing at wide intervals induces very abundant tillering and delays flower formation and ripening, especially in the later-formed tillers. In the interests of plant to plant uniformity it is consequently necessary to limit tillering by sowing fairly closely—at about the intervals customary in agricultural practice. But with close sowing, it is impossible in an eye-survey to decide upon the average stage of development of the population or to select individual plants representative of an agreed standard. Counting the numbers of leaves and tillers and similar determinations, would give a good idea of average (modal) development of the population. If, however, this were done, and the weekly sample were drawn by taking "modal type" plants from all over the bed, for every plant so taken two more (its neighbours) would have to be debarred from all future samples because of the disturbance in their environmental conditions. As has been explained in § IX above, losses due to unavoidable "non-uniformity" were very heavy and it was necessary not to add to them unduly in the drawing of weekly samples. These considerations led to the use of the method, described above, of simply lifting weekly, a whole row of plants. The number of plants used for dry weight determinations was in no week less than ten and in none more than twenty.

(B) *Dry Weight.*

The weekly determinations of dry weight point to one outstanding conclusion. Plant to plant fluctuation is so large that weekly averages cannot possess a reliability compatible with their employment in analysis [*e.g.* increment rates, etc.]. Consequently no more data are published than are required to demonstrate the extent and nature of fluctuation. In Table XXXIV are given the dry weights of the plants constituting the *P* sample lifted on 25. v., a sample which is thoroughly typical. It will

be observed that the range of the distribution exceeds twice the weight of the smallest plant and approximately equals the mean of the sample. This is not attributable to distant "outliers" at the extremes for the dispersion over the range is fairly even. From such a distribution, reliable averages are not to be expected. It is useless to attempt to illustrate
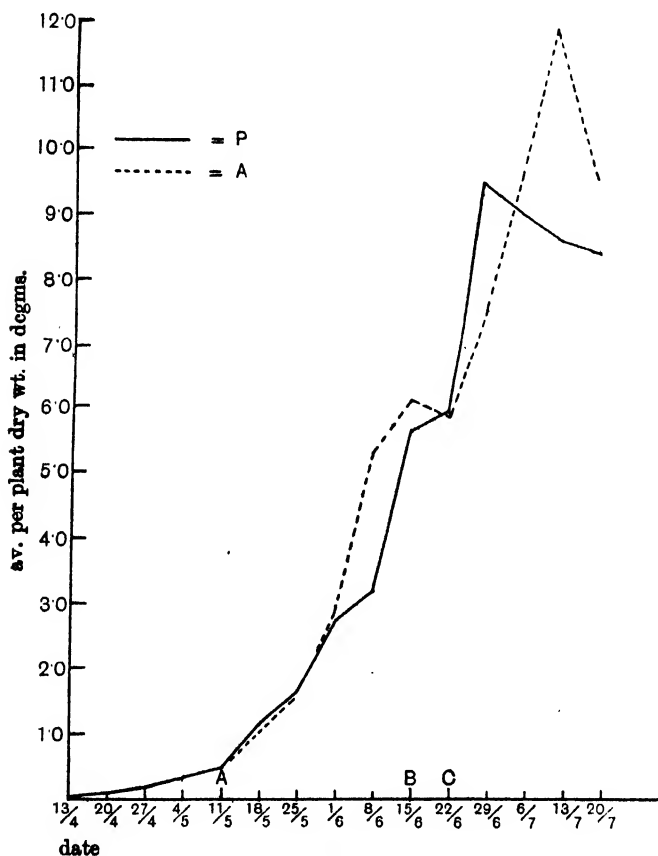
Fig. 4.

the fluctuation by the probable errors of the weekly means for probable errors of widely dispersed samples of 10–20 plants can have no significance.

The weekly averages—low though their reliability must be—afford an interesting side light on the question of "sampling." For this reason they have been given in Fig. 4. In the early stages there is a progressive increase in average dry weight per plant and until 1. vi. the two varieties (*P* and *A*) run closely together. From that date onward, the curves are

irregular and show irregular divergences from one another. On 29. vi. for P and on 13. vii. for A, a decrease in average dry weight begins. Similar decreases have been recorded in other investigations upon dry weight but their probable significance cannot be discussed for this case as the data lack the requisite reliability. But the apparent decrease calls
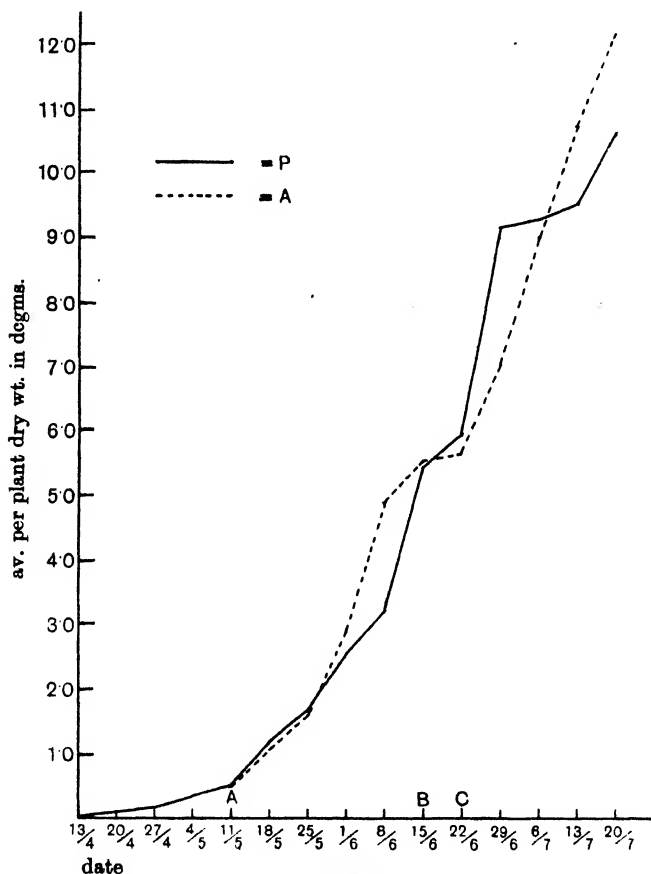
Fig. 5.

attention to the actual distributions of single plant dry weight for the last few weeks. Inspection shows (the data are not published) that in some cases the smallest plant of one week is of greater weight than the smallest of the week following. Let it be granted, provisionally, that this is absurd and, to remove the absurdity, let the lowest plant of the first mentioned week be eliminated from the sample of that week. The process

may be systematically carried out all through, starting from the first week of observation. Correspondingly, starting from the last week, plants may be removed from the upper end of every sample if they are less than the biggest plants of the preceding week. It will be seen that in some cases 2, 3, or more plants may have to be removed from one or both extremes of a weekly range in applying this idea. Such a "correction" of the weekly samples was systematically carried out and the averages of the "corrected" samples calculated. The new averages are plotted in Fig. 5 and it will be seen that the curves now display no break in direction [compare with Fig. 4]. It is quite patent, of course, that the "correction" tends to produce such a change as has resulted and further that if a decrease in dry weight does, as a fact, occur towards the end of the life-cycle, the "correction" must obliterate it. But despite these obvious objections the "correction" has a point of practical interest. The commonly employed method, of sampling by taking plants which appear to be uniform among themselves and representative of the whole population does, in effect, tend to do what the above described "correction" intentionally does. The only real difference is that certain plants (at the extremes of size) are excluded by eye-judgement whereas in the above "correction" they are excluded on the basis of dry weight. Broadly speaking the validity of this objection to the customary method is in inverse proportion to the accuracy with which the eye can assess the "average" stage of development of the whole population. It seems safe to assert that for the freely-tillering cereals grown at the usual close intervals, eye-assessment cannot be practicably accurate.

It is to be understood that in suggesting the parallel between the commonly practised method of sampling and the theoretical "correction," reference is strictly limited to the freely-tillering cereals. The actual numerical data of the more carefully conducted of the investigations upon plants other than these, of itself refutes the suggested parallel.

Clearly, despite the precaution of including only such plants as have enjoyed specified "uniform conditions," samples of the size employed in this investigation are inadequate to the requirements of reliability. The magnitude of plant to plant fluctuation at all stages of growth shows that far larger samples will be necessary. One course would be to draw larger samples and to employ the method of drawing (repeated use of modal classes) described at the end of § VIII (above). This, however, would involve the raising of a very much bigger population of plants and thereby might seriously jeopardise the possibility of confining the growing to a uniform area of ground. Later on, reasons are set forth

for the belief that in work of this kind upon the freely-tillering cereals, the tiller and not the plant should be the unit of observation. This change, rather than the use of much larger samples with its consequent increase in area and in amount of work in weighing, etc., appears the better for future investigation.

<center>(C) Nitrogen Content and Ash Content.</center>

In the early weeks the plants were so small that the complete sample had to be used for N and ash determinations. Later on, it could be divided into sub-samples of 3–5 plants, then single plants were big enough to use, and finally it was possible to determine both the ash and N contents of the same plant. In what follows, the average contents per plant for any week are those calculated from all the plants used for analysis in that week whether in groups of 3–5, etc. or one by one. At all stages, the weekly sample was divided into the greatest practicable number of sub-samples in order to ascertain the extent of fluctuation. This proved to be considerable and it may be illustrated in the case of N by the data of Table XXXIV, col. 6 (for *P* plants lifted on 25. v.). These data are quite typical of the weekly N determinations as a whole. Ash determinations fluctuated rather less.

As with dry weights, so with ash and N contents, the fluctuation prohibits the analytical use of weekly averages. These, nevertheless, merit brief notice. They are given in Fig. 6 and their regularity would suggest that they possess a fair reliability were it not for the great fluctuation known to be displayed by the data on which they are based. It is noteworthy, too, that *P* and *A* display a marked similarity throughout the whole period. To the extent to which the averages are reliable, this similarity suggests that proportionate ash and N contents are not likely to reflect any marked difference of habit or growth between *P* and *A*. It seems safe to make the general inference that for both varieties the contents (per cent.) of ash and N show through the weeks, gradations of very different forms.

From the data, calculations were made of actual average weekly uptake of N and ash per plant. The distributions of these amounts show, for both varieties, violent irregularities. While this must be in large measure attributable to inadequacy of sampling it seems not unlikely that, as a fact, uptake per week is a very fluctuable quantity. Ash uptake is decidedly more regular than that of N but it is unwarrantable to deal with the data in any but these very general terms. N uptake, dependent as it is on both soil moisture and, still more obscure, rate

of nitrification in the soil, may not unreasonably be expected to have an irregular weekly value despite the progressive "growth" of the plant.

The relationship between the percentage contents of N and ash is of some interest and since this, for single plants, in no way depends on "sampling," it may safely receive a critical treatment. In Table XXXV are collected the data for all the single plants which, throughout the investigation, were analysed for both ash and N. These plants were all lifted on or after 1. vi. for prior to that date plants were too small to admit of both determinations. In the table, the plants are serially numbered (col. 1) and arranged in order of N content (per cent.), (col. 2). There are given in addition, ash per cent. (col. 3), number of tillers at the time of lifting (col. 4), date of lifting (col. 5) from which chronological age is determinable, and dry weight (col. 6). To ascertain the relationships subsisting among the five variables (cols. 2–6) it is unnecessary to calculate coefficients of correlation. Preliminary inspection makes it clear that with so few observations (35 plants) the data are not suited to such calculations and, apart from that, interest attaches to the individual plant far more than to the statistical "mass effects" of the population. It is noticeable that accompanying the steady rise in N per cent., there is an irregular rise in ash per cent. and an irregular fall in chronological age—results to be expected from the curves of Fig. 6. Turning to the comparison of single plants, it is immediately apparent that the general trends have no certain application to individuals. For example plants Nos. 10, 11 and 12, or 25, 26, 27 and 28 and so on indicate that N per cent. is not indeed even approximately an index of ash per cent. or dry weight in the case of individual plants. Systematically pursued, the comparison of single plants leads to the following conclusions:

(i) N per cent. is not regularly related to ash per cent.

(ii) N   „   „   „   chronological age.

(iii) N   „   „   „   dry weight.

(iv) As for (ii) and (iii) in regard to ash per cent.

(v) Chronological age is not regularly related to dry weight.

(vi) Tillering bears no regular relation to any of the other variables.

(vii) More complex relationships, *e.g.* dry weight as a possible function of ash per cent. and N per cent. cannot be sought from so small a body of data. But there is little to suggest that more complex relationships would be found to possess any practicable regularity.

In connection with (iii) above it must be mentioned that, in all,

96 plants were used for estimations of N per cent. per plant. The data are not published but they fully confirmed conclusion (iii) above. It was pointed out in § VIII (above) [Time of Flowering] that a clear connection was traceable between time of flowering and early development (on stated chronological dates). Correspondingly, a connection is noticeable between such early development and the dry weight at the time the plants were lifted. This is illustrated by cols. 2–5 of Table XXXIV. In the table the plants (*P* lifted on 25. v.) are arranged
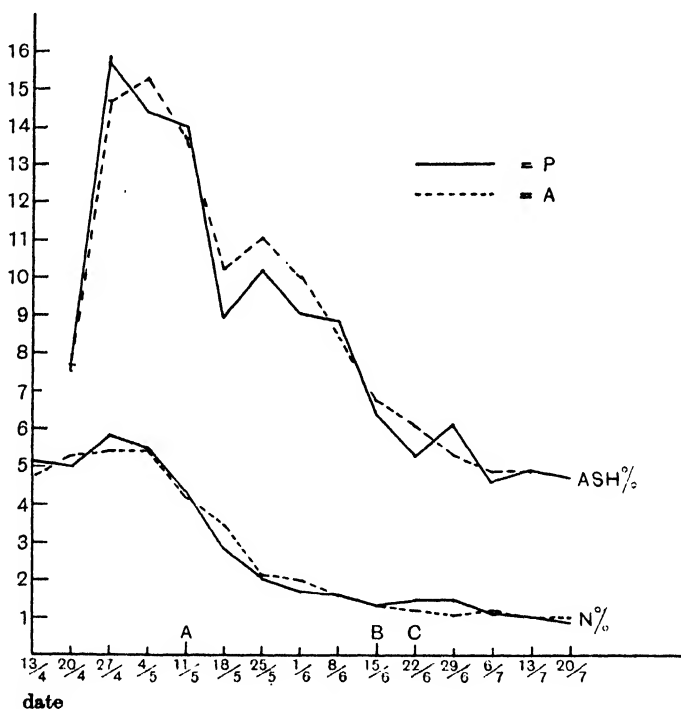
Fig. 6.

in order of dry weight (col. 1) and, as a general effect, the heavier plants are those that in early life had formed relatively the greater number of leaves and tillers. The complete dry-weight data illustrates this fact more extensively but it is not published for the reason that nothing more than a very general effect can be exposed by such a coarsely measured variable as "tillering." [As already pointed out, any tiller, however small, has to be counted and thus number of tillers is a very crude index to the actual development.]

For the purposes of a yield study it is desirable to know whether there is any simply determinable plant attribute which is an index of stage of development attained, *i.e.* of "physiological age." This question has been faced in purely physiological investigations and, on the part of some, the tendency has been to regard percentage of nitrogen as the most reliable index. Much can obviously be urged against it but clearly, for non-tillering plants, it can be well supported by general arguments. The view does not, of course, imply any idea of proportionality but simply that plants having the same N per cent. may be regarded as of the same physiological age. The conclusions which have been outlined [(i)–(vii)] above show that for the *P* and *A* populations concerned here, if N per cent. is to be regarded as an index of physiological age, then none of the other variables of Table XXXV can be so regarded. In the case of ash—part of which is SiO_2 and plays, so far as is known, no part in the metabolism of the plant—it may naturally be urged that there is little expectation of an index of degree of development. Even if this be valid, there remains the fact that ash per cent. bears no regular relation to either dry weight or chronological age. But that it should be related to one or the other of these variables may be argued on grounds comparable to those of the argument in favour of N per cent. as an index of physiological age. It is best to conclude, perhaps, that the physiological significance of all five of the variables, so far as the data of this investigation goes, is not apparent.

(D) *Dry Weight, Nitrogen per cent., and Ash per cent. at Certain Developmental Periods.*

It has sometimes been found that one or more of the quantitative attributes of a plant showed a distinctive relative value at one or more of the commonly recognised developmental periods. For example, for some plants the carbon-nitrogen ratio has always a distinctive value at the time of "flower formation." As recorded in § III (above) [Field Notes on Growth in 1921] efforts were made to determine average values for the whole population of the following variables and the values appeared to be:

(i) Commencement of the formation of the primordia of the ear of the main axis. ["Commencement" here implies that, under the low power of the microscope, in Figs. 4, 5 and 6, lateral spikelets were seen to be marked off from the growing point.]

Date = 11. v. (*A*) in Figs. 4, 5 and 6.

(ii) Emergence of ear of main axis from leaf sheath [this occurs immediately prior to fertilization].

Date = 13. vi. (*B*).

(iii) Fertilization of the florets of the main axis ear completed, and endosperm formation just beginning.

Date = 22. vi. (*C*).

These three dates are marked *A*, *B*, and *C* respectively on the abscissa axes of the curves of Figs. 4, 5 and 6. From Figs. 4 and 5 it may be observed that over the *B–C* period for both varieties there is practically no increase in dry weight whereas increases occur both before and after the period. And from Fig. 6 it is noticeable that the *A* period coincides with the beginning of a very rapid fall in ash per cent. No final conclusion can be reached from data such as that upon which Figs. 4–6 are based but perhaps it is more than should be attributed to coincidence that here, as in other investigations, distinctive points on the distribution of a weight variable occur at definite periods of development. Period *B*—the appearance of the ear from the leaf-sheath wrapping—may at first seem scarcely to deserve to be called a developmental period, and yet it must be borne in mind that this stage is invariably followed by an exceedingly rapid growth and elongation of the top internode of the stem.

(E) *The Results of the Foregoing Sections viewed in Relation to the Phenomenon of "Tillering."*

Among the conclusions reached in the foregoing sections are the following:

(i) In spite of special precautions, the method of sampling employed has failed to give reliable weekly average values. Far larger experimental populations and far larger samples would be required for reasonable reliability.

(ii) For plants of the same chronological age, the percentage contents of N and ash show wide fluctuations.

(iii) The five variables—dry weight, N per cent., ash per cent., chronological age, and number of tillers—appear to show no regular inter-relationship.

The nature of tillering and the inconstancy of the inter-relationship among the tillers of a plant have been fully discussed in §§ VIII and IX (above). In terms of these it is not difficult to suggest an explanation for the series of "negative" conclusions which has been reached. A simple hypothetical case will serve to illustrate the lines of explanation. Two plants each of dry weight x may have each four tillers (main axis = T_0

and three side tillers $= T_1$, T_2 and T_3) and the distributions of dry weight may be:

	T_0	T_1	T_2	T_3
1st plant	0·5x	0·4x	0·05x	0·05x
2nd ,,	0·5x	0·2x	0·2x	0·1x

From Fig. 6, based on complete plants, it is safe to assume that for the individual tiller as age and dry weight advance, there is likely to be a falling off in ash and N per cent. An age difference of a fortnight may bring a considerable falling off (*vide* the decreases for the average single plant between 11. v. and 25. v. in Fig. 6). Consequently the first of the above hypothetical pair of plants, having 90 per cent. of its dry matter in the form of two chronologically "old" stems (T_0 and T_1) is likely to have considerably lower percentages of ash and N than the second, 50 per cent. of whose dry weight consists of relatively young and small tillers. On these lines and without appealing to the influences of soil, etc., it is possible to suggest reasons for great irregularity in the N per cent. : dry weight relationship (and likewise for the N per cent. : chronological age relationship). Further, since the changes of N per cent. : ash per cent. with time are of very different forms (*vide* Fig. 6) it is to be expected that the relationship of these two variables would be very different for the two hypothetical plants. These considerations suggest that the irregularities which have been recorded are inevitable with the freely-tillering cereals and fuller discussion of these plants adds to the probability of this. One or more of the side tillers usually dies considerably before the plant has completed its life cycle. In the *P* and *A* populations this mortality had definitely commenced on 8. vi.—some seven weeks before harvest. As a result the single plant—a colony of units (tillers)—becomes reduced in number of units and the reduction superimposes an effect upon the progressive increase in dry weight of the surviving units.

It is very clear then, that in addition to the difficulties which beset investigations with non-tillering plants on the single plant basis, there are, with the freely-tillering cereal races, other special and formidable difficulties. The importance of studying the physiological inter-relationships of the tillers of a single plant has already been urged (§ IX above). To treat the tiller rather than the plant as a unit of observation seems to hold more promise than a mere increase in the size of the experimental sample and the more so that such an increase both multiplies labour and tends of itself to induce fluctuation.

Special and considerable difficulties owing to the characteristic

"tillering" were to be anticipated. They were anticipated; but that alone afforded no assistance in the formulation of a theory of "yielding power." Definite knowledge was required and this part of the investigation was undertaken with no other expectation than that of acquiring a knowledge of the causes, extent and nature, of inter-plant fluctuation.

§ XI. SOME METRICAL ATTRIBUTES OF SINGLE PLANTS AND OF POPULATIONS.

The work to which this paragraph relates was intended to afford information upon the following matters:

(a) Plant to plant fluctuation under growth conditions calculated to give regularity.

(b) The relationships of yield of grain and straw per plant to other metrical attributes of the plant.

(c) The "migration coefficient" (see below).

(d) The comparative growth of plants at two different spacings.

(e) A comparison of P and A in regard to (a)–(d).

Data were obtained from the plants of Bed 6 (4-inch spacing) and Bed 7 (2-inch spacing) particulars of whose arrangement have been already recorded in § I above. Plants which had not satisfied the conditions of uniformity [Nos. (i), (ii), (iii), (iv) and (viii) of § IX above] were excluded from observation as were those which, at harvest, had less than three ears. This latter step—the exclusion of 1-ear and 2-ear plants—was dictated by limitations of time. Harvesting was a lengthy operation and it was, of course, essential to complete it as rapidly as possible. A preliminary count showed that for all four populations (two varieties each at two spacings) the "modal" class was at or above the 3-ear plant class and it was for this reason that, in reducing the material to be handled, the 1-ear and 2-ear plants were rejected. This artificial limitation of the population prescribes the use to which the data can be put and must be borne in mind in the considerations which follow.

At harvest every plant was uprooted, the root freed from earth, then the whole tied up in a stout paper wrapping, and labelled. Great care was necessary to avoid shedding of grain and breakage of the very fragile leaves and awns. Harvesting was carried out on 28. vii., 29. vii. and 30. vii., the work proving too much for a single day. The plants were stored in the laboratory on lattice shelves well above ground level so that, as explained in § IV above, there was little likelihood of error owing to differential losses of water. In preparing the plants for weighing,

the side tillers were separated from the main stem and their bases carefully freed from particles of soil. The grains were removed, the awns cut off, the number of grains per ear counted, and then the total grains of the plant weighed. The rest of the plant—complete plant less grains and roots—was then weighed as "straw." To maintain the standard of accuracy, all weighings were made to 0·001 gramme but, for the purposes of computation, weights correct to 0·01 gramme were actually used. For the plants of P Bay 7, the length of the rachis of every ear was measured in addition to the other determinations.

The following symbols will be employed:

G = weight of grain ⎫
S = ,, straw ⎬ per plant.
n = number of grains ⎪
T = ,, ears ⎭
m = migration coefficient $= G/\overline{G+S}$
M = arithmetic mean of a frequency distribution.
σ = standard deviation of a frequency distribution.
v = coefficient of variation $= 100\sigma/M$.
Q_1 = lower interquartile of a frequency distribution (*i.e.* the value below which 25 per cent. of the observations lie).
Q_2 = upper interquartile.
$Q_2 - Q_1$ = interquartile range which is given instead of the numbers or curves of a frequency distribution to lessen expenses of publication.
Errors given for means are "standard errors," *i.e.* $\sigma/\sqrt{N}$ (where N = number of observations).
The unit of weight is the gramme.
For the frequency distributions it was necessary to employ class intervals larger than the unit of measurement but the effect of this grouping upon the values of the coefficients of correlation was not sufficient to make it worth while to employ Sheppard's Correction.

A brief explanation of the "migration coefficient" may be necessary for the reason that so much of the work which relates to it is unpublished. It was introduced by Dr E. S. Beaven of Warminster who explained it at the 1914 Meeting of the British Association and, on a subsequent occasion [Beaven (40)] more fully dealt with its use. He found that (*loc. cit.* p. 115): "the migration coefficient for any particular race in any particular season and on any particular soil (which are the only conditions under which we can compare different races) can be calculated by weighing up the entire ripe stems and the entire ripe ears of about 2000 individual plants within a probable error less than 0·2 per cent." The coefficient is defined as "the ratio of dry matter accumulated in the seed to the total dry matter of the plant when fully ripe": thus in terms of the symbols given above $m = G/\overline{G + S}$. This coefficient stands quite alone as an "index

of yielding power" and has been continuously tested by plot experiments directed to the selection of heavy-yielding forms of barley from hybrid progenies.

It seemed possible that the coefficient might, if harvesting and weighing were done with great care, prove to be of service as an "index of yielding power for single plants," *i.e.* the index so greatly needed in plant breeding for dealing with an F_2. As a first step in ascertaining the limits of its application an examination of some 1913 Barley Chess Board Trials data was made. Nine varieties were grown and there were eighteen plots per variety, a plot being one yard square after "trimming" and containing 108 plants. The correlation of G and $G/\overline{G+S}$, *i.e.* yield and migration coefficient for the plots of a variety, was worked out for the nine varieties and the values of "v" were:

$(+0\cdot10)$ $(-0\cdot55)$ $(+0\cdot27)$ $(+0\cdot08)$ $(+0\cdot28)$ $(-0\cdot18)$ $(+0\cdot22)$ $(+0\cdot38)$ $(+0\cdot25)$

These results were not very assuring in regard to the probable applicability of the coefficient to single plants of the ·same variety although they in no way militate against the claims made on behalf of the coefficient for its value in discriminating between different varieties by suitable plot trials. It is to be observed, however, that in harvesting the chess-board plots the care which can be bestowed in single-plant work is impracticable and, moreover, the whole ear (grains, rachis and awns) was weighed as G. It therefore seemed desirable to carry out careful determinations upon single plants. In 1920, as related in §I, plants were grown in flower-pots: but despite careful precautions the inter-plant fluctuation was great and only very general conclusions were reached concerning the migration coefficient. They harmonise with the conclusions to be given in this paragraph but merit no special attention. The growings of Beds 6 and 7 were undertaken with a view to direct determinations of "m" for single plants grown under "uniform conditions" (already described) and harvested and weighed in such a way as to obtain as exact a rendering as possible of the "migration coefficient" (awns were weighed as straw, losses of leaf, grain, etc., were prevented).

The precise statistical and biological significance of the migration coefficient is not, at first sight, clear. Lines of argument readily suggest themselves but it seemed desirable to accumulate numerical data in terms of which the coefficient might be interpreted and an attempt has been made to accomplish this. The inter-relationships of the variables G, S, T, n, and their ratios always suggest themselves as essential in the formulation of a theory of "yielding power." Their determination was a prime object in this investigation.

Expense forbids the publication of the full single-plant data. The essential part of it and the deductions from it are contained in:

Table XXXVI for P and A populations at 2-inch spacing (Bed 7): showing the mean, coefficient of variation, quartiles, and limits of range, of the frequency distributions for various metrical attributes.

Table XXXVII as for preceding table but in the case of P and A at 4-inch spacing (Bed 6).

Table XXXVIII for P and A at both spacings: showing frequency distribution of number of ears per plant.

Table XXXIX for P and A at both 2-inch and 4-inch spacing: showing the correlation of G and S with other metrical attributes of the plant.

Table XL for P and A at 2-inch spacing (Bed 7): showing average values per plant for populations of 3-tiller, etc., plants and ratios derived from these average values.

Table XLI for P and A at 4-inch spacing (Bed 6): showing data corresponding to those of Table XL.

Table XLII. Correlation between number of grains on the whole plant and number on first ear ($= n.T_0$), between number on first ear ($n.T_0$) and on second ($= n.T_1$), etc., for P and A at 2-inch spacing (Bed 7).

The numerical results contained in these tables have now to be discussed and this may be conveniently done in relation to the objects of investigation [(a)–(e)] set forth at the head of this paragraph.

(a) *Plant to plant fluctuation under growth conditions calculated to give regularity.*

Both the conditions of sowing, etc., and the conditions of acceptance of plants for observation, have already been fully described. From an experimental point of view it is important to bear in mind the rigour of these conditions and the labour which their imposition entails. It would be exceedingly burdensome to exercise yet greater precautions and thus the fluctuation exhibited by these populations may, perhaps, be fairly regarded as the least which is to be expected under the conditions of careful field experiments with barley.

At the outset it may be observed from the means, quartiles, etc., of Tables XXXVI and XXXVII that the frequency distributions of G, S, and n are decidedly asymmetrical while those of the "ratios" G/n, etc., are more or less symmetrical. The values of the coefficient of variation (v)

are of some interest in connection with the relative constancy of the attributes G, S, and n. To display these values in simple form the letters G, S, and n are set out below in the order of magnitude of their coefficients of variation:

P—2-inch spacing $G > S > n$

A—2-inch   ,, $G > S > n$

P—4-inch   ,, $G > n > S$

A—4-inch   ,, $S > G > n$

Thus the order of magnitude of the coefficient of variation among G, S, and n is not constant for the four different populations. In some cases G and S differ but little in the value of "v" and the safest conclusion seems to be that $G \eqsim S > n$ the range of values for all three being some 35–40 per cent.

From Tables XXXVI and XXXVII useful information may be obtained as to the size of sample necessary to limit the standard error of a mean value to any desired extent. For example, with the population of A Bed 7 (2-inch spacing), to determine the mean value of G (per plant) to an accuracy of 1 per cent. (of the mean itself) a sample of about 1540 plants would be necessary: for an accuracy of 5 per cent., a sample of 62 plants would suffice. Similarly with the population of A Bed 6 (4-inch spacing) the sizes of sample would be 1408 plants for 1 per cent. accuracy and 56 plants for 5 per cent. accuracy. It is not to the present purpose to examine the sizes of sample for other populations and other attributes but it may be noticed that a population of less than 2000 plants—a size attainable with care in the F_3 family of a wheat or barley cross—suffices to give the mean per plant values to 1 per cent. This generalisation applies, however, only to a population grown and selected according to the specifications of "uniformity" already described.

The "ratio attributes" m, G/n, and n/T must next be considered. For them the coefficient of variation (v) is much lower than for the absolute attributes G, S, and n and it is clear from Tables XXXVI and XXXVII that for all four populations the order of magnitude of v is:

$$\frac{n}{T} > m > \frac{G}{n}.$$

Further consideration of this fact may be postponed until the use of the migration coefficient is considered.

The number of ears formed per plant is shown by the average values of T in Tables XXXVI and XXXVII and the frequency distributions of Table XXXVIII. At both spacings A is superior to P, a fact which it is difficult to harmonise with the slightly greater tillering of P in the

young stage in Bed 6 (cf. Table XII). It might be argued that although (in Bed 6, 4-inch spacing) P formed more tillers than A, yet they were relatively less vigorous. The notes of § III (above) make clear that the dying-off of late tillers was more conspicuous in P than in A and to this extent the argument is supported but it cannot be regarded as proved. The fluctuation in number of ears formed per plant is comparatively small owing, it is believed, to the precautions adopted.

Table XLII casts a somewhat different light upon inter-plant fluctuation. Despite the great range of values of G in all four populations (roughly from 1·5 to 10·5 grammes) there is a significant correlation of $+ 0.6$ between the number of grains borne by the whole plant and by T_0 (the first ear). Similarly the correlations of $n . T_0$ and $n . T_1$, etc., are considerable. It is, in effect, as if plants of all sizes (within a pure line population) developed according to a more or less rigid formula for inter-ear relationships and in consequence the size of the first ear is an approximate index to the size (number of grains) of the whole plant. The biological interpretation of this is, perhaps, that the rudiments of the side tillers are laid down while the main stem is very small and that the primordia of the ears which reach maturity are all developed within a fairly sharply defined period. This interpretation accords with the observations upon ear-primordia formation given in § III above[1].

Each of the experimental populations may be divided into sub-populations according to the number of ears ripened per plant. Tables XL and XLI show the metrical attributes of the 3-, 4-, and 5-ear sub-populations. In many cases the sub-populations are too small to permit of the calculation of the standard errors of means so that any conclusions reached from the data have a validity limited in this respect. The mean per plant attributes—G, S, $\overline{G + S}$, and n given in columns 2–5 of the tables—increase with number of ears for all four populations. This increase was to be expected from general experience but the data of the tables are sufficiently emphatic both to confirm the expectation and to show that the increase is a well-marked one. It follows that for an approximate

[1] Supplementary to these correlations are some results obtained by a class of advanced students working with 20,000 plants from a field crop of Archer barley. The number of grains per ear was counted on the modal-type plants (3 ears). It was assumed that the largest ear (*i.e.* having most grains) was T_0, the next T_1 and so on. Correlations were evaluated between $n . T_0$ and $n . T_1$, etc. To test the validity of these a population of cards was prepared bearing the values of the frequency distribution of number of grains per ear for all the ears of all the 3-tiller plants. The cards were drawn in threes and the highest treated as $n . T_0$ and so on. Correlations were evaluated and they were all considerably less than those derived from the actual plant data. That is to say, the correlations derived from the plants were not invalidated by the assumption that the largest ear was always T_0, etc.

grading of plants for "size" (whether represented by G, S, $\overline{G+S}$, or n) the basis, of number of ears per plant is a perfectly safe one within a pure line. A later paragraph contains a suggestion for making use of this fact in a modified form of small scale yield trial.

The reliability of the values of G, etc. (means per plant for the sub-populations) is of interest. For example, taking the 4-ear population of A Bed 7 it will be found that the sizes of sample necessary to limit the standard error of the mean value of G (per plant) to a certain percentage of the mean itself are:

For standard error = 1 per cent. of mean, size of sample = 459 plants

| ,, | ,, | ,, | = 2 | ,, | ,, | ,, | ,, | = 114 | ,, |

,, ,, ,, = 2 ,, ,, ,, ,, = 114 ,,

,, ,, ,, = 3 ,, ,, ,, ,, = 50 ,,

,, ,, ,, = 5 ,, ,, ,, ,, = 18 ,,

Thus for a given degree of accuracy in determining G (mean per plant value) a far smaller sample is required in dealing with a constant ear-number population than in dealing with the general population. [For A Bed 7 to give 1 per cent. accuracy for G (mean) the general population necessitates a sample of 1540 plants while for the 3-ear population 460 plants suffice.] This difference in size of sample—naturally expected—is so great that it suggests the possibility of dealing only with modal-plant populations in yield trials and § XII (below) is devoted to that possibility.

Although it is not possible to give standard errors for all the values of Tables XL and XLI the following figures indicate that the differences of 3-ear and 4-ear plants at any rate are significant:

3-ear plants A Bed 7 for G ...... $M = 2.92 \pm 0.119$
4-ear plants A Bed 7 for G ...... $M = 4.67 \pm 0.143$.

The interpretation of the change in values of the "ratio attributes" (columns 6–12 of Tables XL and XLI) among the constant ear-number populations is difficult. One significant fact is quite apparent—none of the ratio attributes is constant for all the sub-populations of the same pure line at the same spacing. Thus as among the single plants of a pure line the possibility of constancy in these or any comparable "ratios" does not exist. An "index of yielding power" applicable to single plants will not be found, it thus seems, in the form of any simple "algebraic" attribute. None perhaps was to be expected but the vital need of an "index" in breeding work made it desirable to explore the obvious, even if slender, theoretical possibilities.

Some of the ratios exhibit a similarity in change of values in the passage from the 3-ear to the 4-ear and 5-ear populations. Thus for both P and A at 2-inch spacing G/n attains a maximum for the 4-ear plants although at 4-inch spacing the maxima are for P at 4-ears and for A at 3-ears. G/T behaves similarly and, save in the case of P Bed 7 it always attains its maximum at the same point as G/n. S/n is very similar to G/n while S/T always attains its maximum at the same point as G/T. In spite of these similarities, however, no two of the ratios place the six sub-populations (3-ear, 4-ear, and 5-ear each at two spacings) in the same relative order. The attributes G, S, and n, however, all place the six sub-populations in the same order of merit for both the varieties. Thus the ratio-attributes, whatever their value on the ground of their smaller coefficients of variation, lack the fundamental "grading value" which G, S, and n display.

Finally, the values of G, S, n, G/n, m, and n/T have been evaluated for every plant in all four populations, but it has not been found possible to represent G (for the single plant) as a function of any combination of the other variables with any practicable degree of reliability. In other words, as far as this investigation is concerned, no "index of yield" for the single plant has been found.

(b) *The Relationships of Yield of Grain (G) and Straw (S) per plant to other Metrical Attributes of the Plant.*

It has already been concluded that so far as the data of this investigation is concerned, there is no hope of representing G as a function of other plant attributes in a manner which is closely accurate and which applies to single plants. In this section the relationships of G for a complete population are dealt with in the form of coefficients of correlation.

For every individual plant in all four populations (two varieties each grown at two spacings) G, S, n and T are known. On account of its limited range of fluctuation T cannot be used in correlations in a manner strictly comparable with the remaining attributes. From these attributes, $m = G/\overline{G + S}$, G/n, and n/T have been evaluated for every plant so that six attributes in all are available for the determination of correlations. Other ratio-attributes, *e.g.* S/T might have been evaluated but it seemed unlikely that their employment would materially assist the investigation. From the six attributes, fifteen correlations could be worked out for every population, *i.e.* sixty in all, but of these only 24 are given in Table **XXXIX**. They were selected because of their practical interest.

The remaining 36 would, if determined, widen the knowledge of the relationships but not, it seemed, add to it in principle.

Table XXXIX contains the 24 coefficients of correlation. All are positive and all significant in terms of their own standard errors. In all four populations (with the exception that for P Bed 6 $m > G/n$) the coefficients stand in the same relative order which, writing "m" for the correlation between G and m and so on, may be thus represented:

$$n > S > n/T > G/n > m.$$

The actual values of the coefficients have a significance limited to the varieties, the soil and the season of the experiment; but since some of the values are very high and have a high probability, it seems likely that the correlations which they represent are fundamental. And moreover, the almost complete regularity of the sequence of values in four populations, may be accepted as indicating which, in general, are likely to be high correlations and which low. No conclusive result could be reached without experiments in other years but these results are a useful guide for such.

The six attributes, all correlated with G, may be used as "indexes" to the value of G and from this point of view their coefficients of variation (v) are of importance (see Table XXXVI). In all four populations G/n has the lowest value of v, the values for P at 2-inch spacing being, for example, for the attributes in the above order:

35·09   38·21   19·62   13·85   15·99

Hence for a given size of sample, G/n (mean value) is the most accurately determinable of the six attributes.

To use one of the attributes for calculating G, the equation of the line of regression would have to be employed. Let Y denote the attribute and let g and y denote the deviations of G and Y from their means. Then the equation of the line of regression is

$$g = r \cdot \frac{\sigma_g}{\sigma_y} \cdot y \quad \dots\dots\dots\dots\dots\dots\dots\dots(1).$$

Further let $Sg = \sigma_g \sqrt{1 - r^2}$ so that $Sg = $ the standard deviation of $\left(g - r \cdot \frac{\sigma_g}{\sigma_y} \cdot y\right)$. Now if the regression is nearly linear Sg may be regarded as a measure of the standard deviation of the G array, i.e. of the values of G calculated from those of Y. The suitability of any attribute as a means of calculating G, thus depends upon the value of Sg, i.e. of

$\sqrt{1-r^2}$ where r is the coefficient of correlation of G and Y. For the population cited in illustration above (P Bed 7) the values of $\sqrt{1-r^2}$ are:

n	S	n/T	G/n	m
0·456	0·663	0·733	0·745	0·897

Thus from this point of view, n is again the most suitable variable to use and m the least.

The use of the regression equation may be briefly illustrated by two cases for which the regression equations are transposed from the form of equation (1) above to contain the actual variables (G and Y where Y now is n) instead of their deviations. The equations are:

for P Bed 7: $G = -0.215 + 0.0479\,n$ ......(2),

for A Bed 7: $G = -0.896 + 0.0560\,n$ ......(3).

Applying these results to the means for 3-ear plant sub-populations it is found that:

for P Bed 7: G (mean) = 3·220 (observed)
                     = 3·214 from equation (2),

for A Bed 7: G (mean) = 2·92 (observed)
                     = 2·749 from equation (3).

Thus for mean values such as these, the regression equations afford a fairly accurate index to G in terms of n. In similar fashion G could be evaluated in terms of the remaining five attributes.

For some years past correlations have figured prominently in genetic work. Their application may take two forms first as between mean values for different pure lines and next as between values for a population of plants of the same pure line. The work of Collins[41] on maize illustrates the employment of the first application in order to render distinctions between separate pure lines more certain. Beaven[40] has afforded the outstanding example of the use of the correlation method for selection for "yield" in barley (he finds G and m correlated as among separate pure lines). The second application—as among plants of the same pure line—is the one employed in the investigation dealt with here and it has a somewhat different scope from the first. In theory it may serve three purposes, viz.:

(i) To "characterise" pure lines. Thus the correlation between two attributes may, in all circumstances, be higher in one variety than in another. In Table XXXIX the $G : m$ correlation is higher for P than for A at both spacings. Further test might prove that this difference

held good generally between P and A. Pure lines such as the P and A of this investigation need no correlations to differentiate them but possibly some of the morphologically similar cereal forms might thus be separated.

(ii) To calculate the values of an economically important attribute (*e.g. G*) from some other attributes whose values are observable with greater accuracy and facility.

(iii) To afford information upon biological attributes.

Upon (i) the available data allow nothing more to be said and the amplification of (ii) and (iii) may conveniently be carried out by a discussion of the correlations of G with the six variables $n, S, \dots$ seriatim.

The correlation of G with n—*i.e.* yield of grain per plant and number of grains per plant—being the highest of the series, may be considered first. It has been shown by a simple example how G can be calculated from n: the next consideration that arises is whether this calculation possesses any practical value. This, indeed, is a question that is very pertinent to all such cases of correlation. The coefficient of variation of n is just about the same as that of G and G can be determined by weighing. Nevertheless, the use of n possesses certain advantages. In single plant work it is a much simpler and shorter operation to count n than to determine G by weighing, for the grain must be separated out before it can be weighed. And again, the accuracy of G is greatly at the mercy of fluctuations in water content. Apart from these considerations, any circumstance, such as loss of grains by shedding at harvest, disturbs the value of G just as much as that of n. It seems, then, that for yield investigations upon plants of the same pure line, the use of n as an index to G may have a considerable value. The high correlation of G and n for single plants indicates that as between separate plots of the same pure line, the yield of grain (*i.e.* value of G) per unit area is likely to be closely reflected by the value of n per unit area. Differences in the value of G/n (*i.e.* the average weight of a single grain) may limit the closeness but it has been explained that G/n has a very low coefficient of variation. Plant breeding and agricultural practice in which "yield per unit area" is commonly estimated by eye-judgment, probably in most cases illustrates the fact that for a unit area G and n are highly correlated. The observer instinctively grades as the highest in yield per unit area that plot or field which is most densely "covered," *i.e.*, upon which grains seem most to abound: and it is probable that the observer, particularly with barley, would generally do this even if the plots were of different pure lines. This being so, it is of interest to consider the values of G/n in some of the

well-known commercial barleys. A single example—from the 1921 Report of the Olympia Agricultural Company (30)—will suffice. Five different barleys grown at five centres display a range of 25 per cent. in 1000-corn (dry) weight for the samples of one variety from the different centres and about the same range for the different varieties at any one centre. This is a very high range—as high as is likely to be met in most years—and it doubtless reflects the extreme drought of 1921. Clearly, then, as between different pure lines, n is not a reliable index to G unless allowance be made for the values of G/n, a course which is discussed in § XII below.

The biological implications of a high value of $r_{G.n}$ are best seen by determining the partial correlation $r_{GS(n)}$, *i.e.* of G and S with n constant. For all populations this is low. For example, in the case of P Bed 7 its value is 0·198. From this it follows that G cannot be high for a plant unless n is high, however high the value of S. Now a high value of S would involve, among other things, a great photosynthetic area. Of other circumstances affecting photosynthesis nothing can be said but it seems reasonable to conclude that a great photosynthetic area will imply a potentially great amount of carbohydrate-formation. This, however, will not result in a high value of G for the plant if n be low (as $r_{GS(n)}$ shows). In other words, the capacity for storage—the number of developing endosperms—as well as the capacity for manufacture of carbohydrate, seems to be an important attribute in governing "yield of grain." There is suggestive experimental confirmation for this conclusion. In 1920 and 1921, the alternate spikelets of one or more ears of certain plants were removed as soon as fertilization had taken place. The spikelets which remained developed grain of average size and exhibited no increase from what might at first be regarded as the greater opportunity of acquiring carbohydrate from the food flow. As a result the yield per plant suffered in proportion to the number of spikelets removed, *i.e.*, the number of storage units placed out of action. A crude mechanical analogy may, indeed, be drawn between the developing endosperms of the barley plant and the porters unloading trains in a goods yard. There is a limit to the per head per unit time work accomplished by the porters and, whatever the quantity of goods available, the actual quantity accumulated in the yard will be very dependent upon the number of porters working. Comparative observations on various cereal forms afford further suggestive evidence. Thus in the commercial English wheats Rivet, the heaviest yielder, has a small leaf area compared with Red King the yield of which is relatively

low: but, of course, concerning the qualitative differences of leaf tissue, etc., nothing is known. Rivet is certainly superior in "n."

Nothing very decisive can be said concerning the remaining correlations. That between G and G/n is high and G/n has a low value of v. This suggests that the correlation might have uses comparable with those of G and m as among varieties but it is impossible to project conclusions derived from single-plant pure-line populations into an array of separate pure-line means.

The considerable value of r for G and n/T in all four populations, *i.e.* between yield of grain per plant and average number of grains per ear of the plant, calls attention to a point of some interest. It may be seen from Table XXXIX that G is highly correlated with G/n (average weight of a single grain), n (number of grains per plant), S (weight of straw per plant), and n/T (average number of grains per ear): and further, S and n are highly correlated. It follows from this that G, G/n, n, S, and n/T all reflect, in their values, the "success" with which the plant has grown and matured. Thus they all afford a means of comparing the growth of a well-known standard form in different localities or in different years. In the testing of new forms a standard must always be employed and attributes which afford check indications of the growth of a standard are very valuable. Interest naturally centres on G but fluctuation in water content, high coefficient of variation, loss by birds or by shedding, make G an attribute upon which a form of check is most necessary.

In the case of P Bed 7, the total length of the rachises of every plant was measured [it is denoted in Table XXXIX by Σ rachis] and it proves to be highly correlated with G and S and to possess a variability less than those of both G and S. These facts suggest that it might conveniently, in some investigations, serve as a substitute for S since it is much more accurately determinable. The $S:n$ correlation illustrates what was observed from the young plants—that the ear primordia are laid down at a very early stage of development. Indeed it suggests rather more for it may be argued that the circumstances which control the amount of straw control the number of florets which are actually fertilised [some non-fertile florets are always found in barley at the base of the ear and as a cluster—the endährchen—at the tip].

Reviewing these results in the light of the three purposes which theoretically, as explained above, may be served by the determination of correlations for a pure line population, it may be said that the first purpose—the discrimination of different pure lines—is but poorly served. Although P and A are so readily distinguished by eye, only one correla-

tion $r_{G \cdot m}$ seems to be distinctly different for the two varieties. The second purpose—the evaluation of economic attributes from attributes more accurately and readily determined—is actually realised. G can be calculated from n, and S from n or from Σ rachis; and again G may in some cases be usefully determined from G/n. The remaining possible calculations of G have less value. For certain experimental purposes and as a check or correction in small scale yield trials the calculations rendered possible by these correlations may have a distinct value. If correlations of this kind could be shown to persist as among mean values for different pure lines they would be important indexes of yielding power and as such, of great value in selection. Work within the pure line paves the way for comparative correlation studies, indicates suitable kinds of attribute for investigation, and affords information upon the fluctuation and the necessary size of sample. Biological information—the third theoretical purpose—appears at first sight to have but a small place in the results. Certain plant attributes in both P and A are shown to be conjoint indicators of the degree of development attained by the mature plant. In addition it appears that the environment of the early stages in the life of the plant exercises a comparable effect upon the final yield of both grain and straw. That this would be so might be predicted from the fact that when the leaves are forming and the stem beginning to develop, the primordia of the ears are being laid down. But a mathematical confirmation of this prediction is important for it implies that the attributes G, S, n, etc., chosen for their experimental practicability, are such as to reflect fundamental biological facts, that, in other words, they possess a biological significance. An arbitrary character of uncertain biological significance is unlikely to prove of any value whatever in comparative work—the facts set forth, for example, by Kiesselbach (42) vouch for this—and the study of attributes within the pure line has afforded reliable guidance for inter-varietal work.

(c) *Incidental Observations on the Migration Coefficient.*

It has been explained that the coefficient is defined as $m = G/\overline{G + S}$ and that its use in the comparison of different pure lines is based upon Beaven's experimental conclusion that among pure lines G and m (per unit area values) are correlated. The data available here permit of no inter-line comparisons but to some extent they serve to test the significance of "m" as an attribute of a pure line population.

Beaven (40, p. 115) found that a sample of 2000 plants (representing twenty plots each of one hundred plants) gave a value of m whose

probable error was 0·2 per cent. This result was obtained from "plot-values," *i.e.* m (plot) = G (total for plot)/$\overline{G + S}$ (total for plot) and thus it cannot readily be compared with a value of m which is a mean of the values of m for single plants. With certain assumptions, however, the probable errors of the two values might be expected to be about the same. Actually, as the following data show, the expectation is realised:

for P Bed 7 the p. e. of the mean value of $m = 0\cdot241$ p. c. of the mean

A	„	7	„	„	„	$= 0\cdot199$	„	„	„
P	„	6	„	„	„	$= 0\cdot207$	„	„	„
A	„	6	„	„	„	$= 0\cdot206$	„	„	„

The applicability of m to very small samples—if possible to single plants—is an important consideration. Whether m for a single plant could be held to characterise the field-crop capacity of the pure line is, of course, a matter for separate enquiry: the first question is one of reliability. From Tables XXXVI and XXXVII it may be seen that $100 \, \sigma/M$, *i.e.* the per cent. standard error of m for a single plant is about 13—16. This error is far too high to admit any hope of the application of m in selecting single plants. As a guide to the practicable limits of error in selecting on the basis of small plots it may be noticed that the values of m for separate pure lines given by Beaven (*loc. cit.* p. 114) range from 0·452 to 0·483—a range of some 6·5 per cent. Thus for inter-line selection an accuracy of not less than 1 per cent. seems necessary. To ensure this, the minimum size of sample is 80 plants, a size which is soon attained and readily handled. It must be remembered that this minimum applies only to plants grown and selected for observation with the precautions which have been described but as far as it goes this result is very encouraging. It is not suggested, of course, that final decisions upon yielding power could be reached from such small samples but the first elimination of low-yielding lines might, perhaps, be thus effected.

That m, in comparison with G/n is both more variable and less highly correlated with G, has already been pointed out in section (*b*) above. Despite certain theoretical objections there seems good reason for testing the merits of G/n in inter-line selection. Agriculturally m is a rational index since, being the proportion of dry matter which is stored in the grain, it represents what has been called "seed forming energy." Correspondingly, G/n represents the capacity for storage of a single grain: and since as has been shown G (per plant and probably per acre) depends on n (total number of grains) the "capacity" of the single grain is of

great importance. While it cannot of course be suggested that G/n should displace "m" as an index to selection, it may reasonably be urged that the two should be used in conjunction.

The next matter of interest is to see whether m is much affected by differences of spacing, differences which considerably influence T and G. From Tables XXXVI and XXXVII the following values for the four complete populations are obtained:

P Bed 7 (2-inch spacing) $G = 5\cdot67$: $m = 0\cdot431$
P ,, 6 (4-inch spacing) $G = 4\cdot25$: $m = 0\cdot414$

Difference   1·42   0·017

A Bed 7 (2-inch spacing) $G = 5\cdot12$: $m = 0\cdot437$
A ,, 6 (4-inch spacing) $G = 4\cdot36$: $m = 0\cdot423$

Difference   0·76   0·014

Clearly the proportional difference between means of populations in the case both of P and of A is far less for m than for G. To this extent, therefore, m "characterises" a pure line more accurately than does G since it is more constant for different spacings. The value of this greater constancy in plot trials is readily apparent. Germination failures, losses by wire-worm, etc., may seriously deplete the population of a plot. Thus there remain fewer plants at greater average spacing and in these circumstances, as is shown by the figures given above, the proportional change produced in m will be less than that produced in G. In such a case, therefore, m clearly has advantages which G lacks.

From the figures above it will be seen that for both P and A, m has a higher value for the 2-inch spacing than for the 4-inch. The most obvious difference between the two spacings is in average number of tillers per plant and consequently it is necessary to examine the relationship between this variable and m. Tables XL (2-inch spacing) and XLI (4-inch spacing) contain data for the comparison. For the 2-inch spacing both P and A show a maximum value of m for 3-ear plants while at 4-inch, both show a minimum for 4-ear plants. The number of observations is inadequate to warrant the further pursuit of these facts but by way of illustration of the general principle the values of m for the sub-populations of P Bed 7 (2-inch spacing) may be noticed. They are:

3-ear—$m = 0\cdot427$; 4-ear—$m = 0\cdot443$; 5-ear—$m = 0\cdot434$.

Between the values of m for the 3-ear and 4-ear populations there is a difference of about 3·6 per cent. and this is considerable in relation to the range of values found by Beaven for different pure lines. While it would be unsafe to attach significance to the actual numerical values of the differences, it seems justifiable to conclude that the value of m

is dependent upon the number of ears per plant in the population. If this be true, then the most representative value of m would be that of the modal ear-type sub-population separated out from the complete population. To illustrate this the values of m for P may be noticed:

2-inch spacing complete population $m = 0.431$ } Difference $= 0.017$
4   „   „   „ $m = 0.414$
2-inch spacing modal (3-ear) plants $m = 0.427$ } Difference $= 0.009$.
4   „   „   (5-ear) „ $m = 0.418$

Thus by confining observation to the modal type m has attained a somewhat greater constancy. This, however, cannot be strongly emphasised: it is merely suggestive.

It is concluded that m—independently of its inter-varietal correlation with "yield"—has many of the properties of an "index." Comparative constancy for different plant spacings, low coefficient of variation implying fair accuracy on small samples, fair constancy for separate subpopulations (of constant numbers of ears per plant)—all are properties which make m more amenable to observation than G.

These observations on m are, of course, merely incidental. It must be remembered that the coefficient was introduced and has been used solely upon the plot basis and for inter-varietal comparisons. Table XXXIX shows that the correlation between G and m (per plant basis) is at both spacings higher for P than for A. How reliable such differences are and how prevalent among a range of different pure lines, cannot be here decided. It is, perhaps, a question deserving further attention for theoretically it may imply that inter-pure line comparisons on the basis of m should be limited to such lines as have comparable values of the $G : m$ correlation. That is to say, theoretically, some exceptional pure lines may occur in which "yield" is not as accurately reflected by m as is the case with cultivated pure lines in general.

(d) *The Comparative Growth of the Plants at the Two Spacings.*

In this connection "growth" implies the "end products," the results of growth. Two spacings only were employed as, in the absence of data on the relationship of growth and spacing, it seemed desirable to attempt nothing more than a preliminary indicative investigation.

Tables XXXVI and XXXVII contain the data for comparison of the single-plant averages for whole populations. The value of T (average number of ears per plant) is, for both varieties, greater at the 4-inch spacing than at the 2-inch. Corresponding increases are to be anticipated in some of the other variables. The interspacing differences are set out below in the form: column (1) 4-inch minus 2-inch as a percentage of

the 2-inch value; column (2) the values of column (1) expressed in terms of $1000 = 15.66$ per cent.

Variable	(1)	(2)	(1)	(2)
G	33·41	2133	17·43	1113
S	41·95	2678	22·12	1412
n	31·33	2001	15·66	1000
T	29·22	1866	22·88	1461
G/n	37·94	2423	32·89	2100
n/T	2·01	128	-5·79	-369

The increases of P are, for all the variables, greater than those of A. Denoting the column (1) increase for every variable by the symbol for the variable, the order of magnitude of the increases is:

for P: $S > G/n > G > n > T > n/T$

for A: $G/n > T > S > G > n > n/T$.

These sequences seem to have the following qualitative inferences:

(1) In both P and A, the increase in S in greater than that in G. It is for this reason that the value of the correlation between G and m is different for the two spacings.

(2) G/n, *i.e.* the average weight of a single grain shows the greatest increase in A and the second greatest in P. Of all the A increases it is the one which most nearly equals the corresponding increase in P.

(3) In spite of the considerable increase in G/n which A shows, there is not a correspondingly great increase in G. For P, however, the G increase is high. The reason for this inter-varietal difference is to be found in the increase values of n. For P, the value is much higher than for A both absolutely and in relation to the other increases. That is to say the average P plant responds to greater spacing by a considerable increase in the total number of grains which it produces: the response of A is far smaller. Thus it seems that the P plants are the better able to exploit the larger area (4-inch spacing).

(4) At both spacings T (average number of ears per plant) is greater for A than for P. Both varieties show a considerable increase as a result of greater spacing. It is not therefore in tillering and the production of mature ears that A falls behind P so noticeably in responding to the larger per plant area.

(5) The most informative inter-spacing difference is that in n/T. For P it is the lowest of the increases: for A it is a distinct decrease. Now n/T represents the average number of grains per ear, *i.e.* the "size" of the ear and it has been shown [§ IX and section (*b*) § X—inter-ear correlations] that the successive ears of the plant display a steady decrease in

size. Thus the decrease of n/T in A is to be interpreted as the inability of A compared with P to maintain the size of its later tillers. It is in this way that A, despite its increased tillering, is unable to produce as great an increase in G as that which P produces, i.e. to exploit the larger area as adequately as P. It may, perhaps, be inferred that P and A have different optimum seed rates but the available data are inadequate to the prosecution of this possibility. There is, however, another aspect of the "field behaviour" of the two varieties the consideration of which is permissible here. In every field "gaps" are produced by germination failures, by wire-worm, birds, etc. In the affected areas, surviving plants, enjoying a greater plant area than those in the rest of the field, tiller more freely. In this way they tend to compensate for the loss in yield per acre which a decrease in the total number of plants might involve. What may be called the "compensating power" of a variety is dependent upon ability to produce, when the plant area is increased, a greater number of tillers: it is dependent, too, upon the sizes of these additional tillers. The data which have been given for P and A suggest that, in the circumstances of this investigation, P evinced a greater "compensating power" than A. In other circumstances different results might be reached and it is unsafe to draw any general conclusion upon the relative merits of P and A. It is inferred, however, that from the "yield per acre" point of view, "compensating power" is an important consideration and should receive attention in the selection of new cereal forms. Determinations of single plant values of G would have to be made at two or three different spacings on lines similar to those of the investigation here recorded.

Some of the correlations of G and other variables (Table XXXIX) are significantly different for the two spacings. The $G : m$ correlation has already been discussed from this point of view. In the case of the $G : G/n$ correlation it is to be observed that for both varieties the G/n increase is considerably greater than the G increase, especially for A. This may explain the change in the value of the coefficient of correlation though the expectation might have been that the change would be greater for A than for P. Actually the converse is the case. Similar considerations apply to the $G : n/T$ correlation. Coefficients of correlation in biological data are notoriously difficult to interpret and these changes, produced by differences in spacing, emphasise the limited application which such statistical representations may have.

It has been pointed out that, within a single spacing population, G, S, n, T, G/S, and n/T, may all be regarded as measures of the vigour or success of growth of a single plant. In the passage from 2-inch to 4-inch spacing these joint indexes part company. It may be noticed

that the change in m is only 12 per cent. for P and 8 per cent. for A. These changes are smaller than any of those in the list above except for n/T which, however, has a negative sign for A.

The final stage of the inter-spacing comparison may be carried through by means of the data of Tables XL and XLI—the mean values per plant for sub-populations of 3-ear, 4-ear, and 5-ear plants. It is readily apparent that to predict the difference between, say, the average 3-ear plants at 2-inch and at 4-inch spacing, is impossible. The potential development of any plant is predetermined at the outset by the characters of the seed sown. A seed which, at 2-inch spacing became a 3-ear plant might, had it been sown in the 4-inch bed, have become a 3-ear plant, the ears being larger, or it might have become a 4-ear or 5-ear plant. It is to be expected, then, that some of the 3-ear plants at 4-inch spacing will be bigger (in G, etc.) than some of the 3-ear plants at 2-inch spacing and some, similarly, will be smaller. There is no means of predicting the values of the averages for the corresponding sub-populations at the two spacings. Briefly summarised, the actual results are as follows. In both the varieties, for 3-ear plants, the 4-inch average exceeds the 2-inch in respect of all the variables. This again is true of the 4-ear plants for P but for A the 2-inch average is the greater for all the variables except S and G/n in respect of which the difference is small. This may, perhaps, reflect the tendency of A to respond most markedly to increased spacing by an increase in tillering. And P, on the contrary, may be considered to have shown a tendency to increase n rather than T—a tendency which has already been inferred. The differences in the 5-ear plants are irregular and conflicting. With one exception (A 5-ear plants) the change in G is of the same sign as that in n for the whole of the sub-populations.

(e) A Comparison of P and A in regard to (a)–(d).

The previous sections of this paragraph have already brought to notice some aspects of the comparative behaviour of the two varieties. Briefly, the results of comparison are:

(1) At 2-inch spacing A is greater than P in respect of all the variables (Table XXXVI) except n/T but it has lower coefficients of variation.

(2) At 4-inch spacing P exceeds A in G, n, n/T, and S while A exceeds P in m, T, and G/n. As in (1) A has lower coefficients of variation except in the case of S.

(3) The $G : m$ correlation is lower for A at both spacings.

(4) The "compensating powers" of the two varieties appear to be distinctly different. This fact, explained in full in section (d) appears to have considerable practical importance.

Table XXXIV for P plants lifted on 25. v. and giving:

Column 1 = dry weights (grammes).
    ,,   2 = no. of leaves (x) and tillers (y) in the form $x.y$ on 21. iv.
    ,,   3 = no. of tillers on 28. iv.
    ,,   4 =   ,,   ,,   7. v.
    ,,   5 =   ,,   ,,   25. v.
    ,,   6 = N per cent. (of dry weight) for certain of the plants.

1	2	3	4	5	6
0·8608	3·0	2	3	4	2·26
0·9997	3·0	1	2	2	—
1·0656	3·0	1	2	3	2·47
1·1374	3·0	1	2	4	—
1·2754	3·0	1	2	3	—
1·3745	3·0	1	2	2	—
1·4605	3·0	2	2	3	2·41
1·4822	4·1	2	4	4	—
1·4944	4·2	3	4	5	1·65
1·6597	4·1	2	3	3	—
1·7293	4·1	3	4	4	—
1·8537	4·1	2	4	4	—
1·9738	4·1	3	4	5	—
2·0848	4·1	3	5	5	—
2·1132	4·1	3	5	5	—
2·1329	4·1	2	4	4	2·01
2·1384	4·1	3	4	5	1·76
2·2878	4·1	2	4	4	2·23
2·4592	4·1	3	5	5	1·72

Table XXXV for all the plants which were analysed for both N and ash content (per cent. of dry weight).

Serial no. of plant	N per cent.	Ash per cent.	No. of tillers	Date of lifting	Dry weight (grammes)
1	0·85	4·35	5	20. vii.	7·220
2	0·94	4·65	4	13. vii.	8·868
3	0·98	4·45	4	6. vii.	12·044
4	0·98	4·68	5	20. vii.	12·563
5	1·01	4·37	3	6. vii.	7·525
6	1·01	4·62	4	20. vii.	12·393
7	1·02	5·05	2	13. vii.	6·878
8	1·08	5·10	4	20. vii.	7·289
9	1·09	5·53	6	15. vi.	6·578
10	1·11	4·46	5	6. vii.	12·069
11	1·11	5·28	4	13. vii.	6·761
12	1·11	6·62	4	15. vi.	5·326
13	1·15	4·77	4	22. vi.	6·850
14	1·16	5·39	6	15. vi.	4·413
15	1·22	5·60	4	29. vi.	8·356
16	1·25	5·24	3	6. vii.	7·861
17	1·27	7·21	6	8. vi.	3·664
18	1·29	5·91	4	15. vi.	5·910
19	1·36	4·98	4	13. vii.	8·587
20	1·40	9·17	5	8. vi.	3·657
21	1·48	8·93	4	1. vi.	2·656
22	1·55	5·63	5	22. vi.	7·007
23	1·57	6·19	7	29. vi.	11·546
24	1·60	5·92	7	29. vi.	11·752
25	1·65	10·77	5	25. v.	1·494
26	1·65	9·60	2	1. vi.	1·315
27	1·67	6·99	3	29. vi.	8·484
28	1·69	8·34	2	8. vi.	3·156
29	1·72	8·22	5	1. vi.	3·264
30	1·76	9·51	5	25. v.	2·138
31	1·76	9·30	5	8. vi.	3·153
32	1·78	5·60	4	22. vi.	7·550
33	2·00	9·62	7	1. vi.	4·028
34	2·01	10·67	4	25. v.	2·133
35	2·41	10·97	3	25. v.	1·460

Table XXXVI for P and A populations at 2-inch spacing (Bed 7): showing the mean, coefficient of variation, quartiles, and limits of range, of the frequency distributions for various metrical attributes. P = ordinary type; A = italic.

Attribute	$M \pm$ s.e.	v	$Q_2 - Q_1$	Limits of range upper	Limits of range lower	Number of plants
G	$4{\cdot}25 \pm 0{\cdot}17$	$41{\cdot}43$	$5{\cdot}30 - 2{\cdot}97$	$10{\cdot}55$	$1{\cdot}55$	104
	$4{\cdot}36 \pm 0{\cdot}16$	*$39{\cdot}24$*	*$5{\cdot}23 - 3{\cdot}17$*	*$11{\cdot}55$*	*$2{\cdot}05$*	*116*
m	$0{\cdot}431 \pm 0{\cdot}007$	$15{\cdot}99$	$0{\cdot}48 - 0{\cdot}39$	$0{\cdot}53$	$0{\cdot}24$	104
	$0{\cdot}437 \pm 0{\cdot}005$	*$13{\cdot}21$*	*$0{\cdot}48 - 0{\cdot}39$*	*$0{\cdot}54$*	*$0{\cdot}23$*	*116*
T	$3{\cdot}73$ *	—	—	—	—	104
	$4{\cdot}02$	—	—	—	—	*116*
n	$93{\cdot}25 \pm 3{\cdot}21$	$35{\cdot}09$	$108{\cdot}3 - 72{\cdot}1$	225	35	104
	$93{\cdot}81 \pm 2{\cdot}81$	*$32{\cdot}29$*	*$107{\cdot}2 - 71{\cdot}2$*	*255*	*35*	*116*
G/n	$0{\cdot}0448 \pm 0{\cdot}0006$	$13{\cdot}85$	$0{\cdot}051 - 0{\cdot}039$	$0{\cdot}060$	$0{\cdot}027$	104
	$0{\cdot}0456 \pm 0{\cdot}0005$	*$12{\cdot}09$*	*$0{\cdot}051 - 0{\cdot}042$*	*$0{\cdot}058$*	*$0{\cdot}029$*	*116*
n/T	$24{\cdot}9 \pm 0{\cdot}48$	$19{\cdot}62$	$28{\cdot}6 - 22{\cdot}4$	$33{\cdot}5$	$10{\cdot}5$	104
	$23{\cdot}3 \pm 0{\cdot}37$	*$17{\cdot}12$*	*$26{\cdot}4 - 20{\cdot}4$*	*$31{\cdot}5$*	*$10{\cdot}5$*	*116*
S	$5{\cdot}53 \pm 0{\cdot}21$	$38{\cdot}21$	$6{\cdot}55 - 3{\cdot}99$	$12{\cdot}55$	$2{\cdot}55$	104
	$5{\cdot}74 \pm 0{\cdot}21$	*$39{\cdot}15$*	*$6{\cdot}74 - 3{\cdot}93$*	*$15{\cdot}05$*	*$3{\cdot}05$*	*116*

* Full distributions for number of ears per plant are given in Table XXXVIII.

Table XXXVII for P and A populations at 4-inch spacing (Bed 6): showing data corresponding to those of Table XXXVI. P = ordinary type; A = italic.

Attribute	$M \pm$ s.e.	v	$Q_2 - Q_1$	Limits of range upper	Limits of range lower	No. of plants
G	$5{\cdot}67 \pm 0{\cdot}26$	$39{\cdot}42$	$7{\cdot}05 - 4{\cdot}15$	$12{\cdot}55$	$1{\cdot}55$	74
	$5{\cdot}12 \pm 0{\cdot}19$	*$37{\cdot}53$*	*$6{\cdot}59 - 3{\cdot}65$*	*$11{\cdot}55$*	*$1{\cdot}55$*	*100*
m	$0{\cdot}414 \pm 0{\cdot}007$	$13{\cdot}77$	$0{\cdot}46 - 0{\cdot}39$	$0{\cdot}51$	$0{\cdot}24$	74
	$0{\cdot}423 \pm 0{\cdot}006$	*$13{\cdot}69$*	*$0{\cdot}47 - 0{\cdot}38$*	*$0{\cdot}53$*	*$0{\cdot}26$*	*100*
T	$4{\cdot}82$ *	—	—	—	—	74
	$4{\cdot}94$	—	—	—	—	*100*
n	$122{\cdot}47 \pm 5{\cdot}19$	$36{\cdot}49$	$147{\cdot}11 - 87{\cdot}11$	245	45	74
	$108{\cdot}50 \pm 3{\cdot}90$	*$35{\cdot}91$*	*$136{\cdot}14 - 77{\cdot}3$*	*235*	*45*	*100*
G/n	$0{\cdot}0465 \pm 0{\cdot}0007$	$12{\cdot}62$	$0{\cdot}050 - 0{\cdot}043$	$0{\cdot}066$	$0{\cdot}032$	74
	$0{\cdot}0471 \pm 0{\cdot}0006$	*$12{\cdot}33$*	*$0{\cdot}050 - 0{\cdot}044$*	*$0{\cdot}063$*	*$0{\cdot}028$*	*100*
n/T	$25{\cdot}4 \pm 0{\cdot}49$	$19{\cdot}98$	$28{\cdot}5 - 22{\cdot}7$	$35{\cdot}5$	$10{\cdot}5$	74
	$22{\cdot}0 \pm 0{\cdot}39$	*$17{\cdot}77$*	*$24{\cdot}6 - 19{\cdot}2$*	*$30{\cdot}5$*	*$10{\cdot}5$*	*100*
S	$7{\cdot}85 \pm 0{\cdot}32$	$34{\cdot}85$	$9{\cdot}42 - 5{\cdot}95$	$16{\cdot}05$	$3{\cdot}55$	74
	$7{\cdot}01 \pm 0{\cdot}26$	*$37{\cdot}68$*	*$8{\cdot}55 - 5{\cdot}10$*	*$14{\cdot}55$*	*$1{\cdot}55$*	*100*

* Full distributions of number of ears per plant are given in Table XXXVIII.

Table XXXVIII for P and A at both spacings: frequency distribution of number of ears per plant.

Variety	Spacing	Number of ears per plant 1	2	3	4	5	6	7	8	9
P	2-inch	Plants	Plants	53	33	13	4	—	1	—
A	2-inch	not	not	41	49	15	7	3	—	1
P	4-inch	harvested	harvested	14	19	23	10	1	6	1
A	4-inch			20	23	25	16	9	5	2

Table XXXIX for P and A at both 2-inch and 4-inch spacing: showing the correlations of G and S with other metrical attributes of the plant. The "errors" are standard errors $= 1 - r^2/\sqrt{n}$.

N.B. Σ rachis denotes the sum of the lengths of the rachises of all the ears of the plant.

Correlation (r) of G and	P 104 plants 2-inch	74 plants 4-inch	A 116 plants 2-inch	100 plants 4-inch
m	$+0\cdot441\pm0\cdot079$	$+0\cdot494\pm0\cdot088$	$+0\cdot238\pm0\cdot088$	$+0\cdot255\pm0\cdot093$
G/n	$+0\cdot667\pm0\cdot054$	$+0\cdot354\pm0\cdot102$	$+0\cdot576\pm0\cdot062$	$+0\cdot350\pm0\cdot087$
n	$+0\cdot890\pm0\cdot020$	$+0\cdot947\pm0\cdot012$	$+0\cdot993\pm0\cdot001$	$+0\cdot944\pm0\cdot010$
n/T	$+0\cdot680\pm0\cdot053$	$+0\cdot575\pm0\cdot078$	$+0\cdot695\pm0\cdot048$	$+0\cdot528\pm0\cdot072$
S	$+0\cdot749\pm0\cdot043$	$+0\cdot775\pm0\cdot046$	$+0\cdot809\pm0\cdot032$	$+0\cdot809\pm0\cdot032$
Σ rachis	$+0\cdot818\pm0\cdot032$	—	—	—
Correlation of S and				
n	$+0\cdot778\pm0\cdot039$	$+0\cdot826\pm0\cdot037$	$+0\cdot848\pm0\cdot026$	$+0\cdot845\pm0\cdot029$
Σ rachis	$+0\cdot861\pm0\cdot025$	—	—	—

N.B. For Σ rachis (P Bed 7) the values are:
$$M = 27\cdot402\pm0\cdot819: \quad v = 30\cdot94.$$

Table XL for P and A at 2-inch spacing (Bed 7): showing average values per plant for populations of 3-tiller, etc., plants and ratios derived from these average values. $P =$ ordinary type; $A =$ italic.

No. of ears per ratio T (1)	Average values per plant for population				Ratios derived from the attributes of columns 2–5							No. of plants
T (1)	G (2)	S (3)	$\overline{G+S}$ (4)	n (5)	G/n (6)	G/T (7)	S/n (8)	S/T (9)	n/T (10)	$\overline{G+S}/T$ (11)	m (12)	plants (13)
3	3·22	4·31	7·53	71·6	0·0450	1·07	0·0602	1·44	23·9	2·51	0·427	53
	2·92	3·98	6·90	65·1	0·0448	0·97	0·0611	1·33	21·7	2·30	0·423	41
4	4·64	5·82	10·46	101·2	0·0458	1·16	0·0575	1·46	25·3	2·61	0·443	33
	4·67	6·04	10·72	98·7	0·0478	1·17	0·0612	1·51	24·7	2·68	0·436	49
5	5·95	7·76	13·71	131·5	0·0452	1·19	0·0590	1·55	26·3	2·74	0·434	13
	5·14	6·84	11·97	112·6	0·0456	1·03	0·0607	1·37	22·5	2·39	0·429	15

Table XLI for P and A at 4-inch spacing (Bed 6): showing the data corresponding to those of Table XL. $P =$ ordinary type; $A =$ italic.

No. of ears per plant	Average values per plant for population				Ratios derived from the attributes of columns 2–5							No. of plants
T (1)	G (2)	S (3)	$\overline{G+S}$ (4)	n (5)	G/n (6)	G/T (7)	S/n (8)	S/T (9)	n/T (10)	$\overline{G+S}/T$ (11)	m (12)	plants (13)
3	3·62	5·12	8·74	77·1	0·0469	1·21	0·0664	1·71	25·7	2·91	0·414	14
	3·30	4·17	7·47	66·9	0·0493	1·10	0·0623	1·39	22·3	2·49	0·442	20
4	4·97	7·10	12·07	102·2	0·0486	1·24	0·0695	1·78	25·5	3·02	0·411	19
	4·22	6·05	10·27	86·5	0·0487	1·05	0·0699	1·51	21·6	2·57	0·411	23
5	5·48	7·64	13·12	119·1	0·0460	1·10	0·0641	1·53	23·8	2·62	0·418	23
	5·19	7·14	12·33	109·3	0·0475	1·04	0·0653	1·43	21·9	2·47	0·421	25
6												
	6·21	8·56	14·77	131·6	0·0472	1·03	0·0650	1·43	21·9	2·46	0·421	16

Table XLII for P and A at 2-inch spacing (Bed 7): showing correlation between number of grains on the whole plant and number on first ear ($= n.T_0$); between number on first ear ($= n.T_0$) and number on second ($= n.T_1$), etc. For correlation of (n.whole plant) and ($n.T_0$) the whole population was used for P and for A.

For remaining correlations the 3-ear plants were used for P and the 4-ear for A (these were the modal-plant types for the two varieties). The "errors" are standard errors $= 1 - r^2/\sqrt{n}$.

	Value of r		Number of plants	
Correlation between	P	A	P	A
n.whole plant—$n.T_0$	$+0.632 \pm 0.059$		104	
		$+0.632 \pm 0.056$		116
$n.T_0$—$n.T_1$	$+0.504 \pm 0.103$		53	
		$+0.778 \pm 0.056$		49
$n.T_0$—$n.T_2$	$+0.556 \pm 0.095$		53	
		$+0.602 \pm 0.091$		49
$n.T_0$—$n.T_3$	—		—	
		$+0.508 \pm 0.106$		49

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INVESTIGATIONS ON YIELD IN THE CEREALS[1]. I.

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CONTENTS.

PART II (*concluded*).

§ XII. SUGGESTIONS FOR A MODIFIED PROCEDURE IN SMALL-SCALE CEREAL YIELD TRIALS.

The results of a yield trial may be usefully indicative; they can never be decisive. Geographical differences of soil and climate and seasonal fluctuations, limit the applicability of results to the locality and the year of the experiment. No more can be achieved than to ensure that the data accumulated in the prescribed circumstances have as high as possible a degree of statistical probability. For this, there must be adequate precautions against a variety of factors predisposing to error. These factors may be broadly classified as follows:

(*a*) Soil differences within the locality, *i.e.* major-scale differences as, for example, between the two ends of a 10-acre field.

(*b*) Soil differences within small portions of the test area, *e.g.* as between contiguous areas of 1 square ft. or even as between the areas occupied by neighbouring single plants.

[1] Part I and Part II, §§ I–VI, with Appendices I, II and IV and the appropriate tables appeared in this *Journal*, Vol. XIII. Part 4, October, 1923: Part II, §§ VII–IX, with Appendices III and V and tables in Vol. XIV. Part 1, January, 1924: Part II, §§ X and XI, with tables, in Vol. XIV. Part 2, April, 1924. In every number a complete bibliography has been given, but of the tables only those concerned have been published.

(c) Differences in the seeds sown.

(d) Damage by wire-worm, etc., resulting in the killing off of some plants especially in the very early stages of growth.

(e) Irregularities in light intensity and inter-plant competition owing to irregular seed rate or depth of sowing, etc.

(f) Losses during harvesting.

(g) Irregularities in the water content of the harvested material.

In all forms of trial (a), (e) and (f) necessitate the same kinds of precaution. To allow for (a), duplication of the whole series of plots is commonly practised. By "dibbing" the seed at regular intervals and to a constant depth the influence of (e) may be greatly reduced while for (f) sufficient labour and care in all the operations are adequate safeguards.

There remain (b), (c), (d) and (g). Their influences are lessened by the selection of a "uniform" piece of soil, by careful selection of seed—and with cereal seed eye selection seems the best—and by the adoption of a suitable form of "scatter" of small plots (as in the "chess-board" system). Plant breeding urgently needs a method of testing in which a very small scale will ensure sufficient accuracy for the preliminary grading of long series of segregating types such as the F_3 families from a cross. Reduction of scale demands fresh methods of dealing with (b), (c), (d) and (g).

The plots of any chess-board trial present two features to which inter-plot differences of yield (for a single variety) are attributable: more strictly, by which these differences are reflected. Some plants have died off—usually at an early stage—and so have made no contribution to the "plot yield." Those whose neighbours have died have responded to the increased space which they have enjoyed, by increased tillering and thereby have tended to compensate the plot yield for the loss occasioned by the death of the other plants. Among the plants growing at normal spacing some have grown with conspicuous success, some have done very badly, and so on. Causes (b) and (c)—non-remediable ones— have been responsible for inter-plant differences in areas where normal spacing has been preserved. With such diversity in single-plant growth, the best method of estimating the performance of any variety—its "yielding capacity" as distinct from its "actual yield"—seems open to discussion. "Actual yield" or "plot yield" is found simply by taking the mean of the plot yields for all plots of the variety in a chess-board or other suitable arrangement. The "yielding capacities" of two varieties may fairly be considered to be represented, comparatively, by their

mean plot yields provided the varieties have had equal chances of performing up to their full potentialities. Probably they have had equal chances in a chess-board trial containing say thirty 1 square yard plots of every variety. But for selection work upon, say, 200 F_3 families, such a scale of testing would be impracticable. It would be difficult to handle more than one cross in a year and almost certainly, by handling six crosses and judging F_3 families by eye, better results would in the end be secured. What is required, then, is a means of assessing the "yielding capacities" of different forms from very small samples and with a minimum of labour.

Consideration of this requirement in terms of the single plants of a population, suggests a possible form of assessment of "yielding capacity." Let it be assumed that every plant from a plot has had its yield of grain (G) determined and that the population has been classified according to the value of G. The "modal class" of plant will thus be known. Now the "modal" type of plant is the most commonly occurring type; it represents the degree of development—estimating development as yield of grain—most frequently reached by the variety concerned under the influences of varying combinations of different seeds sown and different "patches" of soil encountered in the plot. In a sense, then, it represents what is to be expected from the variety when an "average seed" is sown upon an "average patch of soil." From this point of view it appears to have some claim to be regarded as representing the "potential yield" or "yielding capacity" of the variety. To accept this view involves the assumption that the yield of the "average (i.e. modal) plant" grown from "average seed" on an "average patch of soil," is indicative of the "field behaviour" of the variety, i.e. of its probable performance when drilled in the customary manner in a field. Field behaviour is dependent upon a number of factors whose action is limited by the circumstances of experimental work. The ravages of birds affect field behaviour but in general the variety that does best when birds are excluded will do best when they are not excluded; and so it is with some other of the "field factors." But in regard to regularity of spacing and depth of sowing, the case is not so simple. However, any objections on this ground to the "modal type" procedure are equally valid against ordinary "chess-board" testing for which the dibbing of seed is always practised.

The precise details of procedure for the modal-plant method of testing need not be fully considered here. By rejecting "end plants" and those with a dead neighbour (as in the work described in the preceding paragraphs) greater uniformity of the plants would result. Such

a step might lead, however, to neglect of the "compensating power" of a variety but separate investigation could overcome this difficulty (*vide* § XI, above). It might, further, be desirable to alternate rows or small groups of rows of the different varieties: this is a matter for later consideration.

If only the modal plants from a plot had to be dealt with, determinations of water content would be simplified. Not only would there be a smaller bulk of material to handle but also these plants would have a smaller range of water content than the complete population.

The essence of the method which has been suggested is the contention that the modal plant is representative of the potentialities, the yielding capacity, of a variety. It is quite impossible to say how closely it would reflect "field behaviour"—data for such a conclusion can scarcely be said to exist in the case of the much used chess-board method of test. But the case for the modal-plant method seems sufficiently good to warrant its consideration as a possible means of effecting a preliminary grading of long series of new hybrid forms.

Labour saving is becoming a vital need in plant breeding and the modal-plant procedure appears to lend itself to this. To weigh the grain from even 100 single plants is fairly laborious and consequently a method involving great numbers of single-plant weighings would be impracticable. In § XI (section B) it was shown that, for both varieties and at both spacings, there was a very high and significant correlation between G and n (weight of grain and number of grains per plant). It would be easier to count the grains on a plant than to weigh them so that with very considerable accuracy the "modal type" (in respect of G) might be found by classifying the plants on the basis of "n" and taking the modal type of this classification. This method would have the further advantage that the frequency distribution of "n" for a population is more regular than that of "G." In consequence, the modal type can be determined with greater precision. Counting grains, however, is fairly laborious and further simplification is desirable. This is offered by the characteristic fact that the number of ears per plant (T) is a good guide to the values of both n and G. To determine the "modal type" for G then, the modal type for T might be found. This would be a simple operation for T has a very limited range of values. A possible and not very laborious refinement would be to determine the modal type for n of the "modal type for T population" and to regard it as the modal type for G of the whole population.

More commonly than not, the modal class of a distribution is not

clearly marked, *i.e.* most distributions are not sharply "peaked." Some difficulty is thus likely to attend the selection of the mode and it might be desirable to take the actual modal class and its two neighbours. This matter ought not to prove a serious obstacle, however.

The data of § XI (*vide* Tables XXXVI, XXXVII, XXXVIII, XL and XLI) make it possible to examine some of the salient features of the modal plant method. A warning, already uttered in § XI, must be repeated here—from all four populations 1-ear and 2-ear plants have been excluded. As a consequence distributions are artificially narrowed but the data, nevertheless, serve the purposes of illustration.

Consideration may first be given to the relative fluctuabilities of the general population and the modal ear-number sub-population. It has already (§ XI) been shown that for A Bed 7 the sizes of sample required to ensure 1 per cent. accuracy for the mean per plant value of G are, for the general population 1540 plants and for the modal (4-ear) sub-population 459 plants.

It is of interest, next, to test the proposed method of obtaining the modal type by classifying for T and then, in the modal class of this classification, determining the modal class for n. The following facts are deduced for A Bed 7 from the data employed in § XI.

(i) For the complete population the modal class for n has a class mean of $n = 94.5$.

(ii) For the modal ear-number (4-ear) population the modal class for n has a class mean of $n = 95.0$.

Thus, for the A Bed 7 population the modal class for n may be reliably found by classifying on the basis of T. As a fact, all members of the modal class for n of the general population are 4-ear plants. And since for (i) and (ii) the modal-n values are identical, the same plants are involved in each population and thus for each population the final modal-n class of plant has the same average value of G per plant. Here then, clearly, the method of determining the modal-n class by means of the more readily observed variable T, is justified. And since the correlation of G and n is very high, this simpler procedure should afford a close approximation to the modal value of G for the whole population.

Finally, a hypothetical case may be discussed in illustration of the suggested method. If there be two plots of the same size, sown at regular intervals with 232 seeds (twice the number of plants in the A Bed 7 population), it may be supposed that in the one every plant survives while in the other 25 per cent. die. Let it be further assumed that the plants which die are all in one half of the second plot. Thus this plot is

equivalent to the A Bed 7 population (116 plants) plus a population of 58 plants whose constitution is that of A Bed 6 (4-inch spacing). The actual A Bed 6 population contained 100 plants. If A-2 and A-4 denote the actual A populations, data for which are recorded in § XI above and $(A$-2$)$ and $(A$-4$)$ denote the gross yields of grain of the populations then:

First plot $= 2\ A$-2; second plot $= A$-2 $+ 58/100 . A$-4.

$\therefore$ total yield of grain of first plot:

$= 2 \times (116) \times$ (average G per plant of A-2) $= 2\ (116)\ (4 \cdot 36) = 1011 \cdot 52$.

$\therefore$ total yield of grain of second plot:

$= 116$ (average G per plant of A-2) $+ 58$ (average G per plant of A-4)

$= 116\ (4 \cdot 36) + 58\ (5 \cdot 12) = 802 \cdot 72$.

Thus the plot yields will differ by about 25 per cent. of the lower.

The modal types for T may now be found. For the first plot the modal type is as for A-2, *i.e.* 4-ear plants for which G (mean) $= 4 \cdot 67$. For the second plot the distribution according to T will be:

$T =$	3	4	5	6	7	8	9
A-2 $=$	41	49	15	7	3	—	1
58/100 A-4 $=$	11·6	13·34	14·50	9·28	5·22	2·90	1·16
Second plot $=$	52·6	62·34	29·50	16·28	8·22	2·90	2·16

Now the average values for G in the case of 4-ear plants—the modal class of the second plot—were for A-2 $= 4 \cdot 67$ and for A-4 $= 4 \cdot 22$.

Thus the mean value of G for the modal class of the second plot is:

$$= \frac{1}{62 \cdot 34} [49\ (4 \cdot 67) + 13 \cdot 34\ (4 \cdot 22)]$$

$$= 4 \cdot 57.$$

The modal-class values of G per plant thus differ by only 0·1 which is roughly 2·2 per cent. of the lower value as compared with the 25 per cent. difference of the total yields of the plots.

It is evident that the closeness of the values of G (mean per plant) for the modal classes of the two plots, is due to the fact that that class, for both plots, is the 4-ear class. Actually, the modal class of A-4 is the 5-ear class but although the A-4 constituent of the second plot raises the mean value of T above that of the first plot, it leaves the modal value unaffected (at $T = 4$-ears). The relatively low yield of the second plot as a whole is clearly due to the poor "compensating effect" of the plants when casualties increase the spacing on one part of it. There is a compensation but it does not suffice to counterbalance the effect of a 25 per cent. loss in number of plants.

The hypothetical case rests upon some considerable assumptions and has been discussed in terms of data which do not permit of incontrovertible argument. As an illustration, however, it indicates that in some circumstances the modal plant, having a greater inter-plot constancy than the total yield, may, apart from other considerations, be regarded as better representing the "yielding capacity" of a variety. Without further specific investigation, however, there can be no decisive conclusion.

The suggestions which have been made are to be regarded in the same light as those in § XI which relate to "compensating power." They are attempts to reduce the scale of yield testing in preliminary breeding work and they seek to find expression for the "field behaviour" of a variety. Until the agriculturist can specify "field behaviour" the plant breeder cannot systematically "breed" for it nor can the physiologist, in whose hands the solution of the "yield problem" ultimately rests, commence its biological interpretation.

§ XIII. THE PERCENTAGE NITROGEN CONTENTS OF THE SUCCESSIVE TILLERS OF THE PLANT.

Investigations of the N content of barley have in almost all cases been made upon the grain for they have been prompted by considerations of "malting quality." The critical work has been that upon the relation between "weight of grain" and N content. Johannsen was the pioneer in this field and he found that for Goldthorpe Barley the grains showed a gradation in weight from base to tip of the ear. And further, the larger grains had the lower N content (per cent.). To this latter finding he attached the reservation that exceptions were not infrequent. Munro and Beaven(17) greatly extended the knowledge of the subject. They confirmed Johannsen's general conclusion, dealing, like him, with samples of grains from the base, middle and top of the ear for a group of ears of one variety. Their work made it clear that "season"—in practice almost synonymous with "maturation"—was a very important factor in the weight : N content relation of grains, and they very clearly explained the grounds on which the experimental relationship might be predicted. Attempts to estimate the N contents of the individual grains upon an ear have been briefly described in Appendix IV. It was clear that despite a strong suggestion of a relationship such as has been described above, deviations were frequently displayed by grains on the same ear. To complete the account of the irregularity of N content in the barley plant it may be mentioned that the percentage is very different in the separate parts of the plant the differences being by no means

constant from plant to plant. A long series of analysis gave results which, in round average numbers were:

Percentage N content to dry weight in:

Awns	= 0·35	Straw (less leaves)	= 0·22
Rachis	= 0·50	Leaves only	= 0·50
Grain	= 1·70		

From these facts it is to be anticipated that the percentages of N in the separate tillers of a plant will be unlikely even to follow the same order of magnitude in all plants. Trial with the single plants of 3-ear, 4-ear, etc., populations confirmed the anticipation. In most of the plants the percentage of N was least, both in grain and straw, for T_0 (main axis), and rose in sequence for T_1 (first side tiller), T_2 ... etc. The very small tillers which had formed no ears (infertile tillers) always had a very high N content.

It was found impossible to carry out the great number of single-plant determinations which would have been required to cope adequately with the fluctuations and in consequence bulk samples were investigated. As a first step, 50 well-grown and matured plants of P were divided up and their tillers thus classified:

(α) large tillers bearing large ears;

(β) poor tillers bearing poor ears (grains few and small);

(γ) infertile tillers.

The results of close duplicate analyses gave the following mean values (N content = per cent. of dry weight):

Class	Straw	Grain
(α)	0·416	1·727
(β)	0·531	1·929
(γ)	1·117	—

The values indicate that the smaller tillers have the higher percentage of N in both grain and straw. Infertile tillers are especially rich in N.

Next, all the tillers of a sample of 7-ear plants were thus classified: (α) big ears, (β) medium ears, (γ) small ears, (δ) very small ears, (ϵ) infertile tillers. The means of close duplicate analyses were, for per cent. N to dry weight:

Class	Average number of grains per ear	Per cent. N in straw	Per cent. N in grain
(α)	32·83	0·359	1·926
(β)	27·93	0·399	1·998
(γ)	21·22	0·537	2·204
(δ)	14·18	0·591	2·038
(ϵ)	—	1·201	—

The average ear size in the different classes may be judged from the average number of grains per ear (column 2).

Similarly for a sample of 6-ear plants, all well grown and well matured:

Class	Average number of grains per ear	Per cent. N in straw	Per cent. N in grain
(α)	32·00	0·388	2·072
(β)	27·50	0·420	2·076
(γ)	16·25	0·643	2·487
(δ)	14·50	1·097	3·646
(ε)	—	1·263	—

These results accord with the previous ones in general form.

The available data, slender though it is, seems to deserve consideration for it indicates that a rise in percentage N content in both grain and straw is in general, to be found in the order T_0, T_1, T_2, etc., for the single plant, the infertile tillers being especially rich in N. "N efficiency," *i.e.* the amount of carbohydrate formed per unit of N absorbed from the soil, may be regarded as one of the aspects of the "field behaviour" of a variety. From the tentative conclusions reached above, it follows that the successive tillers of a plant have diminishing N efficiencies. The more marked the decrease in size in the side tillers the less, therefore, will be the N efficiency of the whole plant. In different varieties the sizes of T_1, T_2, etc., relatively to T_0 are somewhat characteristic and it seems probable that N efficiency may similarly in a broad way, characterise varieties.

It must be realised of course that "N efficiency" as used here is intended to carry no physiological significance; it is simply a numerical representation. In this sense the ideal tillering habit appears to be that which results in the early formation of a few good tillers without numerous, late, impoverished ones. This is in accordance with the conclusions of § IX above but its relation to the requirements of "compensating power" (*vide* § XII) will require careful investigation.

PART III.

A RÉSUMÉ.

The cereal "yield problem" embodies all the possibilities of increasing the output of grain per unit area. Part I is devoted to a general consideration of these possibilities category by category. The production of new varieties or forms of cereal plant is the most directly constructive possibility and improvements on other lines will naturally reinforce whatever is accomplished in this way. Of the methods by which new forms may be obtained hybridization is the most prolific. Its application in plant breeding involves reliance upon eye-judgment and although

the more exhaustive alternative to this is theoretically satisfactory it is impracticable. Despite the obvious merits and the past achievements of eye-judgment, there is a great need for some equally simple but more reliable method. Some have sought for a direct "index" to yielding power in the form of a readily determinable "attribute" of the plant. Only the "migration-coefficient" method elaborated by Dr E. S. Beavan of Warminster appears to hold any promise as an index and this appears unsuited to the complete displacement of "eye-judgment" because of the labour it involves. Assuming a really simple index to be feasible, its discovery will demand more careful study of the characters of the cereal plant than has yet been made.

, In hybridizing to produce varieties of superior yielding power, the ideal procedure would be to "analyse" yield into "components." These "components" are conceived to be the most important of the "plant characters" which control yield. Following upon such analysis there would be a "synthesis" of high-yielding forms by the accumulation into one form by suitable crosses, of the optimum combination of "yield components." Thorough study of plant characters is the line along which yield analysis must be approached and it naturally necessitates experiment in which the single plant and not the acre is the unit of observation. But crops are grown by the acre and therefore single-plant work must be joined by a suitable bridge to the circumstances of per-unit-area growing. Differences of spacing as affecting yield per plant appear to be the best bridge so that all plant characters and the inter-relations they appear to show, must be investigated at a series of spatial intervals. Characters apparently suitable for investigation on these lines can readily be named but knowledge concerning them is scanty. How they fluctuate, how big should be the experimental sample for their investigation, their inter-dependence, the desirability and practicability of possible precautions in growing and sampling to the end that fluctuation may be lessened—all these questions point to a general reconnaissance before the problem of yield analysis is directly attacked.

Many other matters claim attention in connection with this great problem. Root system is the most obscure and perhaps the greatest of these but precise data upon time and method of flowering and the rate of increase of dry weight during life are others which cannot well be overlooked. The extensive occurrence of synonyms and its pernicious results indicate that any plant character of possible diagnostic importance is worthy of investigation. For this reason the juvenile characters of the plant should be included in the list of experimental variables.

For general convenience it seems best to base the preliminary study upon two pure lines of 2-row barley. Reconnaissance and not *ad hoc* experiment seems the only justifiable form of immediate experiment proceeding from present knowledge, a view upon which emphasis must again be laid. Relevant literature is reviewed with no idea of compiling a comprehensive summary but only in so far as it affords comparative evidence or casts light upon the nature and experimental suitability of important plant characters.

Part II is an account of experimental work the scope of which is disclosed by the table of contents prefaced to it. That the results are applicable only to the pure lines, the year, and the general circumstances prevailing must always be in mind. To lessen fluctuation there was a series of precautions which are fully discussed. The chief were:

(i) Selection of seed, sowing on one day and by uniform dibbing, and the exclusion from observation of plants which did not germinate in the "modal" period (of two days) for the whole population.

(ii) A similar exclusion of the plants of end-rows, of the end plants of all rows, and of plants one or both of whose neighbours in the row failed to survive till harvest.

(iii) At later stages and for special purposes, observation was limited to plants which "flowered" on the "modal" day and which produced the "modal" number of ears, etc.

(iv) A separate record was kept for every plant at all stages so that correspondence between development at two or more stages could be tested.

The results are given briefly and seriatim. Both the varieties were grown at two spacings ($12'' \times 2''$ and $12'' \times 4''$). To denote the main axis of a plant the symbol T_0 is used, T_1, T_2, etc., correspondingly serving for the first, second, etc., side tillers. For the whole plant or the single tiller, according to the context the following symbols are used:

G = weight of grain, S = weight of straw,

n = number of grains, m = migration coefficient = $G/\overline{G+S}$,

T = number of tillers.

Appendix I. Despite contrary claims the importance of sowing seeds the "right way up" appears to be negligible.

§ I. The material and method of the investigation are fully explained. In § II the accepted differences between the pure lines—the one of

Archer (*A*) and the other of Plumage (*P*) barley—are described. For two such familiar and characteristic races, the possible descriptions are suprisingly meagre. Field notes on growth form the substance of § III. They serve to check and illuminate the more precise data of subsequent paragraphs.

§ IV. The coleoptile and first green leaf possess distinctive features in *P* and *A* and their diagnostic importance seems clear.

§ V. Of various methods of examining the root system simple spraying away of the soil alone proved helpful. The weighing of roots, theoretically of doubtful worth, is in practice entirely fruitless. Between *P* and *A* a definite difference exists. *A* produces early in life a stronger seminal system while *P* devotes itself sooner and more actively to the adventitious system.

§ VI is concerned with the number of grains upon the ear and with their individual weights in relation to their position. Number of grains is extremely fluctuable and, for *P* and *A*, the inter-varietal difference is readily obscured by inter-seasonal effects. Grain weight in relation to position on the ear has a fairly definite pattern for ears of all sizes in any one pure line. But local unconformities on the individual ear make it impossible to employ this "pattern" in analytical work. A comparison of the "pattern" of the "average ears" of *P* and *A* for ears of the same number of grains reveals a difference which appears to be, while quite characteristic, of some importance in regard to yield. Number of grains, among ears of the same pure line, is a safe index to relative weight of ear. To a less extent this is true of the average weight of a single grain.

§ VII. Tillering. Three weeks after germination, despite the "precautions" employed, very considerable inter-plant differences were manifest. At this time the populations were sharply concentrated round the conditions "three leaves and no tillers" or "four leaves and one tiller." These appeared to be definite stages of morphological development. At every count of tillering, for both varieties, and for both spacings, the frequency distributions showed marked regularity. Standard errors of means were small and in the worst set of data a difference of unity between the mean numbers of tillers of the two pure lines would have been significant. For so fluctuable a character as tillering, accuracy of this standard is very satisfactory and it repays the labour which the precautions involve. Between *P* and *A* the difference at all stages was not significant, a result confirmed later by the numbers of mature ears formed per plant.

Appendix III is a review of the question of "tillering."

§ VIII. Time of flowering, concerning which there is a brief review of published experimental results in Appendix V. "Flowering" was taken as the stage at which the awns had emerged one inch from the leaf sheath. Time of flowering is a very sharp "varietal" character and at both spacings A was a little earlier than P. The times for the individual tillers of a plant are definitely correlated, the date for T_0 thus being an index to those for T_1, T_2, etc. Between the dates of flowering of well and poorly developed plants as between those for the individual tillers of a single plant, the intervals were very small and quite disproportionate to the differences of age and of development in earlier stages. Almost every tiller of every plant in all the experimental populations, flowered within a week. The differences, though small, were quite positive and they reflected in a singularly regular way, the differences of earlier stages of growth. In fact by selecting sub-populations which exhibited different degrees of advancement on the first date of observation, corresponding differences could be shown to be present at all the important subsequent stages. As a practical application of these results a method has been suggested for drawing uniform and representative samples at successive stages in work upon dry-weight increase or upon the products of the mature plant.

Appendix IV explores the details of ear attributes. It is shown that, in experiment, to weigh the whole ear (grain plus awns plus rachis) is unsafe and further that in small scale work upon grain weight, etc., dry-weight determinations are necessary. The proportionate weight of the adherent paleae is very fluctuable and sufficiently so to invalidate observations based upon small samples unless its effects are determined and corrected. It seems to be indicated that, save in special circumstances, the total weight and number of the grains of the ear are the only practicable variables in observations upon ear characters.

§ IX. Nothing is known of the physiological inter-relationship of the tillers of a cereal plant. As a first step towards their investigation a study was made of their attributes—G, S, n, etc.—in the mature state. Sub-populations of plants were drawn in each pure line which satisfied a very comprehensive set of specifications of "uniformity." Even in these, inter-tiller relationships were very inconstant. No explanation can be advanced to account satisfactorily for this result and it seems that the actual physiological inter-relationship of the tillers will have to be explored. Among the questions to be dealt with is the possibility that translocatory interchange of salts or of elaborated substances can take place among the tillers of a plant. Possibly the result described is

to be interpreted as a reflection of the extreme arbitrariness, or biological indirectness, of "yield" in the form of weight of grain.

§ X. "Yield" is an expression or end-product of growth. Inter-varietal differences of yield may therefore be revealed or interpreted by growth studies. The tillering habit of cereal plants renders acute the difficulty of "sampling" which besets such studies in all plants. This investigation aimed at nothing further than an exploration of the "sampling" difficulty as a preliminary to further work. For various reasons, determinations of N per cent. and ash per cent. were made and samples were drawn at weekly intervals. One or two rows of plants were lifted and all plants rejected which had not satisfied certain require-ments of "uniformity" of conditions. No other method of sampling is readily possible with cereal plants. Fluctuations in dry weight were exceedingly great and it is shown how very dependent deductions may be upon the form of sampling employed. For the single plants dry weight, N per cent., ash per cent., number of tillers, and chronological age are quite disharmonious and thus no such attribute is acceptable as an index to "physiological age." The curves showing the progressive dry weights of the average plant of the week, though only limited reliance can be placed in them, display at the time of flowering and fertilization a distinct "check" such as has characterised other similar results. In the light of the phenomenon of tillering most of the difficulties and disharmonies can be explained and the desirability of a modified method of sampling (*vide* § VIII) is again indicated.

§ XI is a comprehensive study of the values of G, S, n, m and T per plant as well as of their inter-relationship for both varieties at both spacings. Of sixty possible correlations thirty have been evaluated as these appeared to suffice as indications of the value of this method.

(*a*) In all the populations the correlation of G per plant with the other variables had an order of magnitude which may be represented by writing for the correlation the symbol for the other variable. The order then is:

$$n > S > n/T > G/n > m.$$

As the correlations are high, positive, and fully significant this constancy of sequence is fundamental.

(β) For calculating G in terms of other variables, the use of "n" gives the most accurate results and that of m the least. On biological grounds as well as statistically, a high value of "n" seems the prime essential to a high value of G whether per plant or per acre.

(γ) Total length of rachis per plant is highly correlated with G, S, and n and is less fluctuable than S.

(δ) Compared with the labour it involves, the evaluation of coefficients of correlation has a limited value in yield studies. Nevertheless some of the correlations, particularly that of G and n, have valuable practical applications and are of some biologic interest.

(ϵ) The migration coefficient (m) is slightly different for sub-populations of 3-ear, 4-ear, etc., plants but its constancy is much greater than that of G and thus the claim for its use in selection work receives some support. It is possible that as between members of a group of similar barley varieties it may have an application which fails if members of different groups are treated.

(ζ) For both P and A the increase of S from 2-inch to 4-inch spacing is greater than that of G. Consequently the value of the $G : S$ correlation is dependent upon the spacing of the plants. The response of P to the greater spacing is more generous than that of A in so far as G is concerned. This is due to the fact that A, while at both spacings a more freely tillering form than P, fails to increase as rapidly in "n." In other words its successive tillers more quickly decrease in size below the main axis. This is at once evident from the values of n/T, i.e. number of grains per tiller.

(η) The coefficient of variation, viz. $100\,\sigma/M$ ($\sigma =$ standard deviation, $M =$ mean) had a value of 35–40 per cent. for G, S, and n. To determine the mean per plant value of G to 1 per cent. the size of sample must be 1540 plants: and to 5 per cent. a sample of 62 plants is requisite. These sizes are for samples drawn in accordance with the specifications of uniformity already detailed.

(θ) Ratios such as G/S are naturally less variable than the attributes which compose them. The order of the coefficient of variation was in all cases $n/T > m > G/n$ and G/n shows the highest correlation to G of these three ratios.

(ι) The number of grains on T_0 is correlated with the numbers on T_1, $T_2 \ldots$ and also with the total number borne by the plant. The coefficient $\doteqdot$ $0\cdot 6$ to $0\cdot 7$ so that despite individual fluctuations a tendency to uniformity of inter-tiller relationship as judged by "n" is proved to this degree.

(κ) The value of G may be broadly gauged from that of T. In the A population at 2-inch spacing the mean values of G (per plant) were:

$2\cdot 92 \pm 0\cdot 119$ for the combined 3-ear plants,

$4\cdot 67 \pm 0\cdot 143$ for the combined 4-ear plants.

By observing plants having all the same number of tillers, percentage accuracies of 1 per cent. and 5 per cent. are derivable from much smaller samples than are requisite in dealing with the general population. The actual sizes are for 1 per cent. 459 (instead of 1540) and for 5 per cent. (18 instead of 62).

(λ) The simple ratio attributes G/S, n/T, etc., are all different in value for the sub-populations characterised by different numbers of tillers per plant. Moreover, no two of the six ratio attributes tested, places in the same order of magnitude the different tiller-number sub-populations. Thus in comparing different pure lines on the single plant basis it is essential to confine observation to the "modal" tiller-number sub-population of each line. In no case can close comparison be of value if made in terms of general populations.

(μ) For the single plant it appears impossible to represent G as a function of one or a combination of the other plant variables explored.

§ XII suggests a modified procedure for yield trials on a very small scale. Substantially it rests upon the contention that the yielding power of a variety should be judged by the modal value of G (per plant) for a population. By using the correlations of § XI a shortened practical application of this method seems possible.

§ XIII. The percentage of N in the tillers of a plant is usually in the order $T_0 < T_1 < T_2 \ldots$ for both grain and straw. Small, non-ear-bearing tillers have a very high percentage. Thus the "nitrogen efficiency" or amount of dry matter formed per unit of nitrogen used, decreases in succession through the tillers from T_0. This may be regarded as one aspect of the field behaviour of a cereal variety and the results confirm the finding of an earlier paragraph—that the production of a few tillers very early in the life of the plant with the suppression of late tillering is the ideal habit of the high-yielding cereal plant.

Several lines of further investigation are opened by the results which have been reviewed. In a field as vast as that which the "yield problem" covers, a single line cannot be expected to lead directly to a solution. The general conclusion is that the form of the single plant at different spacings is to be regarded as the immediate consideration and that it should be judged in the first instance on the basis of total number of grains. Comparative work on varieties of markedly different yielding powers is called for since, in the light of the character inter-relationships which have appeared, this may perhaps be expected to disclose the nature of such differences. Simple continuation appears to be requisite in connection with juvenile characters and the root, while

for increase in dry weight the difficulty of sampling needs further investigation for which the lines suggested (modal type selection) may prove suitable. Reduction of labour is vital to yield studies owing to their extensiveness and the results of the detailed investigations afford an indication not only of the size of sample requisite for specified accuracy but also of the limitations to the value of the more important determinations of ear and other plant characters. It seems probable that in further investigation the use of precautions of the kind described will be amply repaid.

Table XXXVI for P and A populations at 2-inch spacing (Bed 7): showing the mean, coefficient of variation, quartiles, and limits of range, of the frequency distributions for various metrical attributes. P = ordinary type; A = italic.

Attribute	$M \pm$ s.e.	v	$Q_2 - Q_1$	Limits of range upper	lower	Number of plants
G	$4\cdot25\pm0\cdot17$	$41\cdot43$	$5\cdot30 - 2\cdot97$	$10\cdot55$	$1\cdot55$	104
	$4\cdot36\pm0\cdot16$	*$39\cdot24$*	*$5\cdot23 - 3\cdot17$*	*$11\cdot55$*	*$2\cdot05$*	*116*
m	$0\cdot431\pm0\cdot007$	$15\cdot99$	$0\cdot48 - 0\cdot39$	$0\cdot53$	$0\cdot24$	104
	$0\cdot437\pm0\cdot005$	*$13\cdot21$*	*$0\cdot48 - 0\cdot39$*	*$0\cdot54$*	*$0\cdot23$*	*116*
T	$3\cdot73$ *	—	—	—	—	104
	$4\cdot02$	—	—	—	—	*116*
n	$93\cdot25\pm3\cdot21$	$35\cdot09$	$108\cdot3 - 72\cdot1$	225	35	104
	$93\cdot81\pm2\cdot81$	*$32\cdot29$*	*$107\cdot2 - 71\cdot2$*	*255*	*35*	*116*
G/n	$0\cdot0448\pm0\cdot0006$	$13\cdot85$	$0\cdot051 - 0\cdot039$	$0\cdot060$	$0\cdot027$	104
	$0\cdot0456\pm0\cdot0005$	*$12\cdot09$*	*$0\cdot051 - 0\cdot042$*	*$0\cdot058$*	*$0\cdot029$*	*116*
n/T	$24\cdot9\pm0\cdot48$	$19\cdot62$	$28\cdot6 - 22\cdot4$	$33\cdot5$	$10\cdot5$	104
	$23\cdot3\pm0\cdot37$	*$17\cdot12$*	*$26\cdot4 - 20\cdot4$*	*$31\cdot5$*	*$10\cdot5$*	*116*
S	$5\cdot53\pm0\cdot21$	$38\cdot21$	$6\cdot55 - 3\cdot99$	$12\cdot55$	$2\cdot55$	104
	$5\cdot74\pm0\cdot21$	*$39\cdot15$*	*$6\cdot74 - 3\cdot93$*	*$15\cdot05$*	*$3\cdot05$*	*116*

* Full distributions of number of ears per plant are given in Table XXXVIII.

Table XXXVII for P and A populations at 4-inch spacing (Bed 6): showing data corresponding to those of Table XXXVI. P = ordinary type; A = italic.

Attribute	$M \pm$ s.e.	v	$Q_2 - Q_1$	Limits of range upper	lower	No. of plants
G	$5\cdot67\pm0\cdot26$	$39\cdot42$	$7\cdot05 - 4\cdot15$	$12\cdot55$	$1\cdot55$	74
	$5\cdot12\pm0\cdot19$	*$37\cdot53$*	*$6\cdot59 - 3\cdot65$*	*$11\cdot55$*	*$1\cdot55$*	*100*
m	$0\cdot414\pm0\cdot007$	$13\cdot77$	$0\cdot46 - 0\cdot39$	$0\cdot51$	$0\cdot24$	74
	$0\cdot423\pm0\cdot006$	*$13\cdot69$*	*$0\cdot47 - 0\cdot38$*	*$0\cdot53$*	*$0\cdot26$*	*100*
T	$4\cdot82$ *	—	—	—	—	74
	$4\cdot94$	—	—	—	—	*100*
n	$122\cdot47\pm5\cdot19$	$36\cdot49$	$147\cdot11 - 87\cdot11$	245	45	74
	$108\cdot50\pm3\cdot90$	*$35\cdot91$*	*$136\cdot14 - 77\cdot3$*	*235*	*45*	*100*
G/n	$0\cdot0465\pm0\cdot0007$	$12\cdot62$	$0\cdot050 - 0\cdot043$	$0\cdot066$	$0\cdot032$	74
	$0\cdot0471\pm0\cdot0006$	*$12\cdot33$*	*$0\cdot050 - 0\cdot044$*	*$0\cdot063$*	*$0\cdot028$*	*100*
n/T	$25\cdot4\pm0\cdot49$	$19\cdot98$	$28\cdot5 - 22\cdot7$	$35\cdot5$	$10\cdot5$	74
	$22\cdot0\pm0\cdot39$	*$17\cdot77$*	*$24\cdot6 - 19\cdot2$*	*$30\cdot5$*	*$10\cdot5$*	*100*
S	$7\cdot85\pm0\cdot32$	$34\cdot85$	$9\cdot42 - 5\cdot95$	$16\cdot05$	$3\cdot55$	74
	$7\cdot01\pm0\cdot26$	*$37\cdot68$*	*$8\cdot55 - 5\cdot10$*	*$14\cdot55$*	*$1\cdot55$*	*100*

* Full distributions of number of ears per plant are given in Table XXXVIII.

Table XXXVIII for P and A at both spacings: frequency distribution of number of ears per plant.

Variety	Spacing	Number of ears per plant								
		1	2	3	4	5	6	7	8	9
P	2-inch	Plants	Plants	53	33	13	4	—	1	—
A	2-inch	not	not	41	49	15	7	3	—	1
P	4-inch	harvested	harvested	14	19	23	10	1	6	1
A	4-inch			20	23	25	16	9	5	2

Table XL for P and A at 2-inch spacing (Bed 7): showing average values per plant for populations of 3-tiller, etc., plants and ratios derived from these average values. P = ordinary type; A = italic.

No. of ears per plant	Average values per plant for population				Ratios derived from the attributes of columns 2–5							No. of plants
T	G	S	$\overline{G+S}$	n	G/n	G/T	S/n	S/T	n/T	$\overline{G+S}/T$	m	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
3	3·22	4·31	7·53	71·6	0·0450	1·07	0·0602	1·44	23·9	2·51	0·427	53
	2·92	*3·98*	*6·90*	*65·1*	*0·0448*	*0·97*	*0·0611*	*1·33*	*21·7*	*2·30*	*0·423*	*41*
4	4·64	5·82	10·46	101·2	0·0458	1·16	0·0575	1·46	25·3	2·61	0·443	33
	4·67	*6·04*	*10·72*	*98·7*	*0·0478*	*1·17*	*0·0612*	*1·51*	*24·7*	*2·68*	*0·436*	*49*
5	5·95	7·76	13·71	131·5	0·0452	1·19	0·0590	1·55	26·3	2·74	0·434	13
	5·14	*6·84*	*11·97*	*112·6*	*0·0456*	*1·03*	*0·0607*	*1·37*	*22·5*	*2·39*	*0·429*	*15*

Table XLI for P and A at 4-inch spacing (Bed 6): showing the data corresponding to those of Table XL. P = ordinary type; A = italic.

No. of ears per plant	Average values per plant for population				Ratios derived from the attributes of columns 2–5							No. of plants
T	G	S	$\overline{G+S}$	n	G/n	G/T	S/n	S/T	n/T	$\overline{G+S}/T$	m	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
3	3·62	5·12	8·74	77·1	0·0469	1·21	0·0664	1·71	25·7	2·91	0·414	14
	3·30	*4·17*	*7·47*	*66·9*	*0·0493*	*1·10*	*0·0623*	*1·39*	*22·3*	*2·49*	*0·442*	*20*
4	4·97	7·10	12·07	102·2	0·0486	1·24	0·0695	1·78	25·5	3·02	0·411	19
	4·22	*6·05*	*10·27*	*86·5*	*0·0487*	*1·05*	*0·0699*	*1·51*	*21·6*	*2·57*	*0·411*	*23*
5	5·48	7·64	13·12	119·1	0·0460	1·10	0·0641	1·53	23·8	2·62	0·418	23
	5·19	*7·14*	*12·33*	*109·3*	*0·0475*	*1·04*	*0·0653*	*1·43*	*21·9*	*2·47*	*0·421*	*25*
6	—	—	—	—	—	—	—	—	—	—	—	—
	6·21	*8·56*	*14·77*	*131·6*	*0·0472*	*1·03*	*0·0650*	*1·43*	*21·9*	*2·46*	*0·421*	*16*

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THE DETERMINATION OF THE NUMBER OF BACTERIA IN SOIL.

II. METHODS FOR THE DISINTEGRATION OF SOIL AGGREGATES AND THE PREPARATION OF SOIL SUSPENSIONS.

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(With Six Text-figures.)

IN an earlier paper the author has described some experiments which showed that a vibratory treatment of a soil suspension resulted in a disintegration of soil aggregates which resisted the action of ordinary shaking, the method being considered more especially in relation to its application to the determination of the number of bacteria in soil [20]. As the problem involved appeared to be fundamentally identical with that of the preparation of a suspension of a soil sample for mechanical analysis, it was decided to postpone further bacteriological work till after the effects of vibration had been examined physically.

The question of the preliminary treatment of a sample of soil for mechanical analysis has now become of greater importance, as a result of recent advances in analytical methods. Great interest was displayed in the subject both in England and in America some ten years ago, but since then little has appeared in English until recently when Joseph and Martin [7] advocated the adoption of the method of Beam [2, 3]. On the other hand a considerable amount of work has been done on the Continent which appears to have been overlooked, doubtless owing to the difficulty and delay experienced in obtaining literature from abroad.

Atterberg [1], who makes use of Beam's Method, mixes the weighed sample of soil in a small round-bottomed porcelain dish with as much water as is required to give a thick paste. This is carefully worked with a stiff brush so as to disintegrate the soil aggregates as thoroughly as possible, and *then gradually diluted,* the working with the brush being continued during this operation.

At a meeting of the "Internationalen Kommission für die mechanischen und physikalischen Bodenuntersuchung" held in Berlin in October

1913 (18), a resolution was passed to the effect that the question required further investigation, more especially in a comparison of the methods of shaking and of triturition. The investigations of Hissink, which were briefly reported at this meeting, are discussed later.

Novák (13) describes the method in general use in Germany and admits that it is not satisfactory.

Richter (16) compares several methods and, on the whole, prefers Hissink's modification of the method of Beam (5, 18).

Kappen (8) draws attention to the flocculating and coagulating effect of ferric hydroxide.

Ehrenberg and van Zyl (4), in an investigation of the finer crumb structure of a soil, compared the effects of shaking samples in their natural moist condition and after air-drying. They found that prolonged shaking (up to 9 hours) with distilled water gave smaller quantities of the clay fraction in the dried samples than in the fresh samples. It is surprising that they obtained such well-marked differences, as one would have expected the effects of air-drying to have disappeared to a large extent during the process of separating the fractions by Atterberg's Method.

Koettgen (9) gives a general account of the methods in use, and advocates shaking so as to eliminate the personal error involved in triturition.

Nolte (12) compares the effect of boiling, and also gives the results obtained by van Zyl in a comparison of triturition with a pestle, the finger, and of shaking.

Oden (14) compares various treatments and concludes that the method of Beam-Atterberg is satisfactory, provided that the paste is either allowed to settle or is centrifuged so as to admit of a second careful treatment with the brush. The diluted suspension in $N/100$ ammonia is then shaken in a shaking machine for 24 hours.

Elsewhere Oden (15) ascribes alleged variations in many of the properties of clays to the presence of aggregates.

Hissink (5) has recently published a full account of his investigations which were first reported at the Berlin conference. It would appear that a fairly satisfactory degree of disintegration is attained by means of the brush method of Beam. Hissink separated the clay fraction by means of water. The residue was again worked up with the brush and the clay again separated by means of water, this operation being repeated ten times, after which only traces of clay remained. Further, he found that a preliminary treatment with dilute acids causes a reduction in the clay content. From the data given it would appear that this effect is due to

a coagulation of the smaller particles into aggregates, and not to a solvent action of the acid.

König and Hasenbäumer (10) report experiments in which they compare the effects of shaking for 1, 2 and 3 hours with triturition in water, with boiling in dilute ammonia, and with boiling followed by triturition. They conclude that shaking alone is not sufficient and recommend boiling for 1 hour followed by triturition. The differences obtained are much smaller than those found by Hissink.

Joseph and Martin (7) advocate the use of sodium carbonate solution in place of ammonia, and a camel-hair brush for puddling the clay instead of a rubber pestle. The period of shaking is also reduced.

Robinson (17) shakes for 2 to 4 hours in an end over end shaker (rate not given), using sodium carbonate for raw clays, but ammonia for soils containing much organic matter.

Jennings, Thomas and Gardner (6) find that 2 hours' shaking with sodium carbonate solution is insufficient to deflocculate certain soils and that oven-dried soils deflocculate more slowly than air-dried soils.

Zunker (22) shakes for $4\frac{1}{2}$ hours in an end over end shaker with $N/10$ ammonia.

Since in the newer methods of analysis it must be assumed that all aggregates have been disintegrated in the preliminary treatment, the problem of obtaining a satisfactory suspension is of paramount importance. In the older (beaker) methods where the fractions are separated out and collected, it is, apart from the saving in time, a matter of indifference as to whether disintegration of aggregates is completed during the preliminary treatment or whether it is brought about gradually during the separation of the clay fraction, providing that it is complete. The work of Hissink would seem to throw grave doubts as to whether this is always as complete as is generally believed. This would explain the disparities between the analytical results of different workers working on the same soil to which the author has drawn attention elsewhere (19).

If the suspension is required for mechanical analysis alone, the duration of the preliminary treatment is merely a matter of convenience, but if bacterial counts are contemplated the time factor cannot be neglected. In addition, the use of ammonia, boiling or any other treatment which would exert a sterilising action is obviously prohibited.

Scope of present work.

In the earlier work the soil suspension was subjected to a vibratory treatment by means of the clapper of an electrical bell, and suffered from

the disadvantage that neither the frequency nor the amplitude of vibration was known. For further work it was considered necessary to have an arrangement by which these could be controlled and varied at will. It was also considered desirable that the strength of blow delivered should be capable of measurement in some way so as to at least define the actual conditions. An apparatus has been designed and made which promises to fulfil most of these requirements, and is described below. A certain amount of work has been done, which, although of a preliminary nature, clearly gives distinct evidence that a vibratory treatment is of service in the disintegration of soil aggregates.
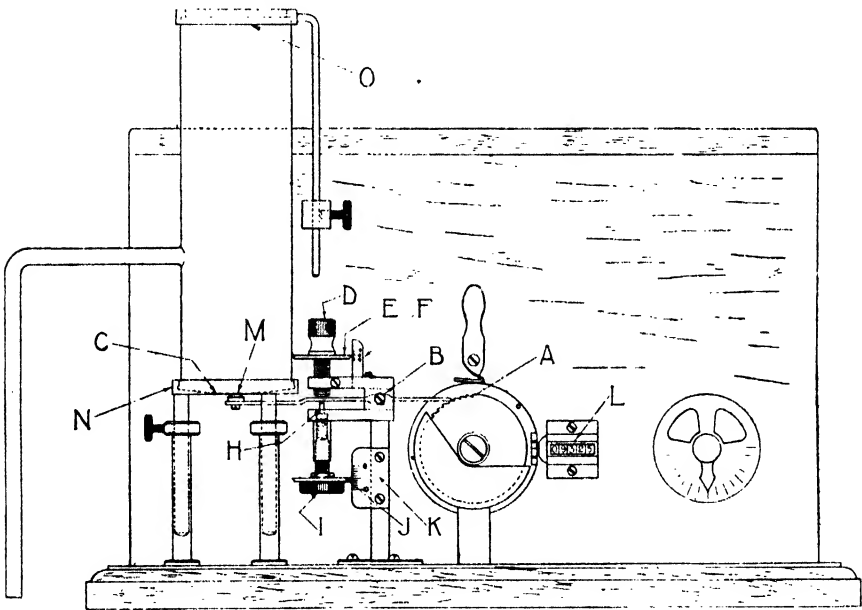
Fig. 1. The Vibrating Apparatus.

Description of the Vibrator.

This apparatus was made by Messrs W. G. Pye & Co. to generate a known number of vibrations at the base of a glass cylinder (Fig. 1). The motive power consists of a massive double drum gramophone motor which has a range of speed of from 80 to 110 r.p.m. approximately. Mounted on the main spindle of this motor is a hardened steel toothed wheel A which operates a lever pivoted at B, which in turn delivers the vibrations to the celluloid diaphragm at the base of the glass cylinder C. The amplitude of this lever is governed by the micrometer screw D, and the movement can be read on the scales E and F. The strength of

blow is governed by the spring plunger H, which in turn is governed by a screw I, the tension being measured on scales J and K. Three wheels A are supplied having 25, 50 and 100 teeth respectively, so giving a large range of vibrations which can be calculated from the r.p.m. of the motor, this being obtained by the revolution counter L fitted at the side of the toothed wheel A. Three striker weights M are supplied weighing 1, 2 and 5 grammes respectively, this also varying the strength of blow. The table N is made adjustable, giving the required latitude to the glass cylinder when setting up the apparatus. Mounted at the top of the glass cylinder is a clamp O which rigidly holds the cylinder in position during the course of the experiment.

Adjustments and Control of Amplitude.

The movable stop D is adjusted so that the lever just makes contact with the toothed wheel A while the motor is running, and the readings on the scales E and F noted. The stop D is then raised by an arbitrary amount (h_1), and the table N is then adjusted so that the striker weight M just makes contact with the celluloid membrane. It is important that the liquid should have been placed in the cylinder before this is done, since the downward pressure of the liquid in the vessel distorts the membrane to an appreciable extent. The stop D is then again raised by a definite amount (h_2), which must not be so great that the lever ceases to make contact with the stop. Hence the amplitude of the point on the lever opposite the stop is equal to $(h_1 + h_2)$, and since the distances of the pivot from the stop and from the striker weight can be measured, the amplitude of the striker weight can be calculated. This involves the assumption that the lever does not bend appreciably while it is vibrating. It cannot be assumed that the amplitude of vibration of the membrane is equal to that of the striker weight; it is probably considerably less. On the other hand it is presumed that these adjustments are sufficient for the present to give conditions which are clearly defined and which are reproducible.

Strength of Blow.

This will be governed by:

    (i) Velocity of the striker at the instant of impact, and this depends on:

        (*a*) The distance of the striker at its lowest position, *i.e.* at the instant at which it is released from a tooth, from the membrane.

        (*b*) The pressure exerted by the spring plunger H.

        (*c*) The mass of the striker weight.

    (ii) The mass of the striker weight.

Up to the present this aspect of the apparatus has not been considered, care only having been taken to maintain uniform conditions. It is thought that it should be possible to calibrate the arrangement here described so that the conditions may be exactly defined, but this requires further investigation. A difficulty has arisen in connection with the spiral springs used in the spring plunger *H*, as they become permanently shortened as a result of the hard treatment to which they are subjected.

AN EXPERIMENTAL STUDY OF DISINTEGRATION BY THE VIBRATION METHOD.

I. RESULTS EXPRESSED AS SUMMATION PERCENTAGES OF "SEDIMENT WEIGHTS."

For obvious reasons measurement of the degree of disintegration of soil aggregates under different treatments cannot be made by the beaker method of mechanical analysis. Further, for the preliminary work, the employment of either the "accumulation curve" method of Oden (14), Wiegner (21) or the depth-concentration method of Robinson (17) appeared unnecessary; the former necessitates a somewhat tedious mathematical treatment and the latter may present experimental difficulties with very dilute suspensions. Since the percentage of the finer fractions will increase with the efficiency of the disintegration treatment the following method was tentatively adopted as suitable.

The Sediment Weight Method.

Two gramme samples of the soil were vibrated for definite periods at various amplitudes and frequencies in 100 c.c. of a 0·025 per cent. solution of sodium carbonate. The glass cylinder, which bore a mark at the 10 cm. level, was then filled up to the mark with the sodium carbonate solution, and after inverting two or three times to break up the muddy cake that had been formed at the bottom of the vessel, the turbid liquid was allowed to settle for 30 seconds, and then carefully decanted into a glass tube, the lower end of which was closed by a celluloid cap. These tubes were about 40 cms. high and about 5 cms. in diameter, and bore a mark at the 30 cm. level measured from the cap. Rubber bands were used to ensure a water-tight joint as with the vibrator vessel. The vibrator vessel was again filled up to the mark with the solution, mixed by inverting as before, and again decanted after 30 seconds' sedimentation. These operations were repeated. It was found that three washings were sufficient to remove the greater part of the finer particles, and it

was desired that the treatment should be as brief and as gentle as possible in order to avoid any further disintegration of the aggregates and also to avoid an excessive dilution of the suspension.

The cap was then removed from the vibrator vessel and the residue washed into a weighed porcelain crucible, together with any particles that remained adherent to the walls of the vibrator vessel. The surplus liquid was poured off and the crucible and its contents dried in the steam oven for 24 hours, and weighed after cooling in a desiccator.

The turbid liquid in the large tube was made up to the 30 cm. mark with sodium carbonate solution, mixed by inverting several times and then allowed to settle for 5 minutes. The supernatant liquid was then removed by siphoning through a platinum rose into a filtering flask under reduced pressure. By starting the siphon about half a minute before the appointed time and keeping the rose just below the surface of the liquid, it was found possible to complete the operation at the desired instant and to remove the liquid to within a few millimetres of the cap without disturbing the sediment. The cap was then carefully removed and the remainder of the liquid added to that in the flask. A fresh cap was fitted and the liquid in the flask returned to the tube, and again made up to the mark if necessary. The sediment on the cap was washed into a small crucible, dried and weighed.

This procedure was repeated for longer periods of sedimentation, the height of the column being increased if necessary so as to give a convenient time for siphoning, as shown in Table I.

Finally the particles still remaining in suspension were precipitated by the addition of acid, collected on the cap, washed into a crucible, dried and weighed.

Table I.

Height of column cm.	Time of sedimentation sec.	h.	m.	s.	Ratio $\dfrac{\text{Depth}}{\text{Time}}$	Log ratio $\dfrac{\text{Depth}}{\text{Time}}$
10	30	0	0	30	·33	$\bar{1}$·5227
30	300	0	5	0	·10	$\bar{1}$·0
30	900	0	15	0	·033	$\bar{2}$·5
30	3,000	0	50	0	·010	$\bar{2}$·0
30	9,000	2	30	0	·0033	$\bar{3}$·5
30	30,000	8	20	0	·0010	$\bar{3}$·0
30	90,000	25	0	0	·00033	$\bar{4}$·5
32·4	324,000	90	0	0	·00010	$\bar{4}$·0
34·2	342,000	95	0	0	·00010	$\bar{4}$·0
36	360,000	100	0	0	·00010	$\bar{4}$·0

It will be obvious that the earlier sediment will contain the bulk of the coarse particles and aggregates, and that the more effective the

disintegration treatment, the smaller will be the weight of these sediments, and the greater the weight of the later ones. If we regard the original suspension as composed of a large number of fractions, each having a definite concentration, it is a simple matter to express each of the sediments in terms of the original partial concentrations of these fractions, but one cannot readily deduce the actual proportions of the fractions originally present. However, on plotting the summation curve of the sediment weights, a very manageable type of curve is obtained, by which the degree of disintegration attained can be readily compared (Fig. 2).

EXPERIMENTAL.

Material employed.

In some preliminary experiments the effects of vibration on a soil that had been stored in the laboratory in an air-dry condition for about two years were examined. In order to obtain as uniform samples as possible this was passed through a 1 mm. sieve and the fine portion rejected. The coarse portion was then passed through a sieve made from the wire gauze used with Bunsen burners. This had square meshes approximately 2·5 mm. by 2·5 mm. The coarse portion which did not pass through this sieve was gently crushed with a wooden pestle in a mortar, and again sieved as before. In this way a large quantity of material was obtained in the form of hard dry grains averaging about 2 mm. in diameter.

As will be seen later, this soil, a Wenlock Shale from near Shrewsbury, is quite exceptional; the fractions Fine Gravel and Coarse Sand obtained by the beaker method are found on microscopic examination to resemble a miniature conglomerate. Since this probably represents one of the most resistant materials that is likely to be encountered, it was thought that it would serve as a good test of the various treatments.

Table II.

	Mean %	Standard deviation
Fine gravel ...	12·7	2·4
Coarse sand ...	5·2	0·75
Fine sand ...	8·4	1·6
Silt	26·8	2·3
Fine silt ...	22·7	1·2
Clay	12·5	1·4
Moisture	1·64	1·16
Loss on ignition	7·20	0·99
Calcium carbonate	0·10	0·06

The mean results of mechanical analyses by the beaker method of the 19 original samples from which the above composite material was

obtained are given in Table II, and that of the composite itself after thorough trituration in a mortar with a rubber pestle will be found in the Appendix.

The Influence of Frequency.

Two gramme samples of the material described above were weighed out and dropped into 100 c.c. of a 0·025 per cent. solution of sodium carbonate in the vibrating vessel, and then vibrated at various frequencies, for 10 minutes in Series I and for 20 minutes in Series II. The liquid was not stirred. The 1 gramme striker weight was used, and the amplitude of the lever at the stop was 0·50 mm.

The resulting suspension was then examined by the method described. The summation percentages of the sediment weights were as follows:

Table III.

	The Influence of Frequency								Various Treatments			
	Series I					Series II			Series III			
Lab. No	(10)	(15)	(16)	(18)	(19)	(12)	(17)	(20)	(24)	(26)	(27)	(25)
	Vibrated for 10 minutes at					Vibrated for 20 minutes at			Hand-shaken only	Triturated only	Triturated and vibrated for 10 mins.	Triturated in ammonia (3 weeks)
$\mathrm{Log}\,\dfrac{\text{depth}}{\text{time}}$	2050	2773	3905	4855	5490	2086	4920	7940				
$\bar{1}$·5	97·0	98·8	98·4	99·4	99·8	100·6	97·6	98·6	99·7	98·8	98·4	92·9
$\bar{1}$·0	18·2	19·0	24·4	29·5	37·5	26·0	37·6	26·6	73·4	80·1	84·0	86·2
$\bar{2}$·5	11·8	12·7	16·0	20·3	26·8	17·0	27·2	17·9	51·5	55·5	59·0	66·6
$\bar{2}$·0	7·6	8·3	11·2	13·6	17·2	11·8	19·0	12·0	33·2	36·0	39·7	48·4
3·5	4·9	5·3	7·2	8·7	10·9	8·3	13·2	7·7	21·1	21·9	25·4	34·1
$\bar{3}$·0	Not determined									15·0	17·9	23·7
4·5	3·1	3·7	4·8	5·5	6·9	5·2	8·6	4·8	11·3	9·6	11·7	15·7
$\bar{4}$·0	1·1	1·5	2·0	2·2	2·3	1·9	3·1	1·8	4·4	6·0	7·6	10·2
Ppt'd res.	0·5	0·6	0·9	1·0	1·0	1·0	1·6	0·8	2·2	3·4	4·7	6·5

In the first series the degree of disintegration increases regularly with the frequency. On the other hand in the second series a still higher frequency is less effective. In other experiments it was found that a greater effect was produced with frequencies of about 5000 or 10,000 vibrations per minute than with intermediate ones. This is probably due to resonance, and may also possibly be connected with the natural period of the membrane itself. It is also probable that the amplitude complicates the question, as it is doubtful whether the full amplitude was attained in No. 20.

Various Treatments.

Two gramme samples were subjected to the following treatments and the suspensions examined as before:

(24) Shaken by hand as vigorously as possible for 10 minutes, in a small conical flask of about 250 c.c. capacity.

(26) Triturated gently to a cream with a porcelain pestle in a porcelain mortar, avoiding grinding as far as possible.

(27) As in (26), followed by 10 minutes' vibration at 10,000 vibrations per minute. 1 gm. striker weight. Amplitude of lever at stop 0·3 mm.

(25) Triturated in a small beaker with dilute ammonia, at intervals daily for 3 weeks with a pestle made by mounting a rubber bung on a glass rod.

The results are given in Table V.

Hand-shaking is clearly a distinct advance on vibration alone, but is not so effective as triturition, while a further vibration, even for 10 minutes, results in a further destruction of aggregates. Assuming that three weeks' triturition with ammonia effects a complete reduction to prime particles, it seemed as if there was a possibility of this result being attained in a reasonable time by a combination of triturition and a rather longer vibration.

Table IV.

	Series IV. Vibration after triturition							Series V. Vibration after triturition Vibrated 1 hour at 10,000 per minute							
Lab. No.	(60)	(61)	(62)	(63)	(64)	(65)	(66)	(70)	(71)	(72)	(73)	(74)	(75)	Mean	S.D.
Log $\frac{\text{depth}}{\text{time}}$	Time vibrated (minutes)														
	10	20	30	40	50	60	80								
$\bar{1}$·5	98·8	95·9	95·7	97·7	94·4	97·3	96·7	98·4	100·2	100·6	97·1	98·9	101·3	99·4	1·4
$\bar{1}$·0	77·9	87·1	91·3	84·1	86·2	92·1	85·9	85·7	89·2	90·7	88·8	92·3	90·5	89·5	2·1
$\bar{2}$·5	54·5	63·8	68·6	60·9	64·3	66·6	63·9	57·0	60·8	65·4	64·5	70·2	66·3	64·0	4·2
$\bar{2}$·0	35·8	45·6	44·1	39·5	46·7	44·0	43·9	37·1	38·9	43·3	43·0	50·2	45·4	42·9	4·3
3·5	23·9	30·6	30·9	25·3	30·8	28·8	30·2	26·5	25·0	28·5	28·0	35·7	29·1	28·8	3·3
$\bar{3}$·0	16·6	20·5	20·8	17·5	20·5	19·4	21·0	17·2	16·2	17·3	18·1	26·4	19·5	19·1	4·3
$\bar{4}$·5	9·6	12·9	12·6	10·8	12·5	11·3	13·6	9·0	9·5	10·9	10·8	19·2	12·6	12·0	3·4
$\bar{4}$·0	5·2	8·1	5·0	5·5	6·6	6·3	8·1	5·6	5·5	6·8	6·6	13·5	8·9	7·8	2·8
Ppt'd res.	2·2	3·9	3·1	3·3	3·8	3·7	4·8	3·2	3·2	4·2	3·8	9·0	6·6	5·0	2·1

Triturition followed by Vibration.

In Series IV the striker weight was 5 gm., the amplitude of the lever at the stop 0·50 mm. and the frequency 5000 vibrations per minute. The results obtained, which are given in Table IV, are by no means satisfactory. It is clear that 10 minutes is not sufficient, but no other conclusion could be drawn.

In Series V an attempt was made to determine the amount of agreement that would be given by a set of six parallels. A mechanical stirrer
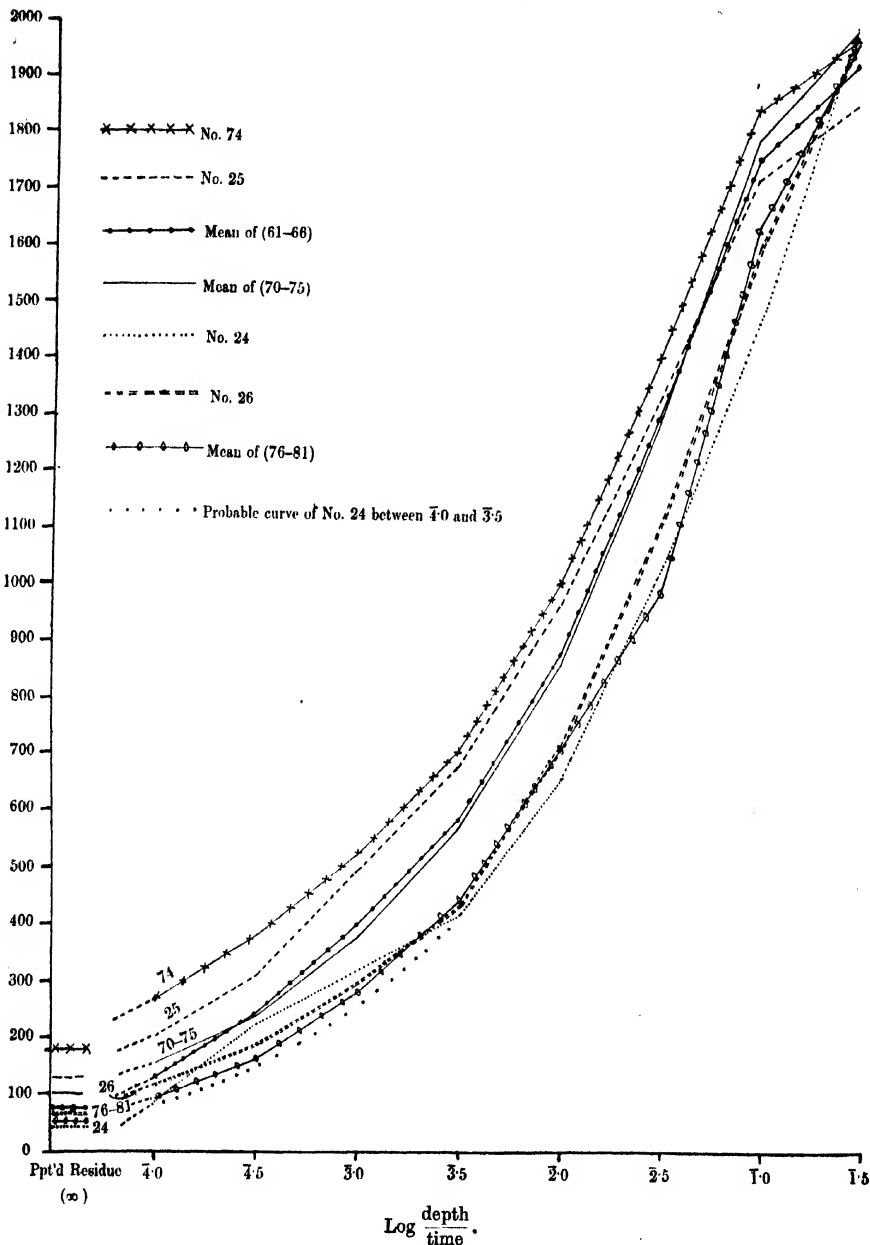
Fig. 2. Comparison of treatments by the Sediment Weight method.
(N.B. Curve of No. 24 between $\bar{4}\cdot0$ and $\bar{3}\cdot5$ is not strictly comparable, as sediment for $\bar{3}\cdot0$
was not determined.)

was used to prevent the accumulation of a thick cake on the membrane. The frequency was raised to 10,000 vibrations per minute. The 2 gm. striker weight was used, the amplitude was 0·5 mm. It will be seen that a very fair agreement is obtained with Nos. 70 to 73, but No. 74, a very turbid suspension, has obviously undergone more disintegration than was attained with any of the previous experiments, with the possible exception of No. 25, in which there is a loss of 7 per cent. No. 75 was almost as good a suspension, a fairly close agreement being found between the sediments after ignition (*e.g.* the precipitated residues weighed 0·129 and 0·112 gm. respectively). At the time, no explanation could be found for the increased disintegration.

Table V.

Series VI. Vibration after trituration
Vibrated 1 hour at 4000 vibrations per minute

Lab. No. Log $\frac{depth}{time}$	(76)	(77)	(78)	(79)	(80)	(81)	Mean	S.D.
1̄·5	98·3	97·0	98·4	98·6	99·0	98·8	98·4	0·6
1̄·0	82·6	80·1	81·6	83·5	80·8	82·3	81·9	1·1
2̄·5	54·1	54·0	48·8	54·3	59·9	55·8	54·5	3·3
2̄·0	34·6	35·1	32·5	36·6	39·7	35·5	35·6	2·2
3̄·5	21·7	21·2	20·5	23·2	25·1	22·4	22·3	1·5
3̄·0	14·6	13·4	13·2	15·1	15·7	13·3	14·2	1·0
4̄·5	7·9	8·4	7·6	8·3	9·1	8·0	8·2	0·5
4̄·0	4·5	4·9	4·5	4·7	5·3	4·7	4·7	0·3
Ppt'd res.	2·5	2·8	2·4	2·8	3·2	2·8	2·7	0·3

Series VI is a repetition of the last experiment at a lower frequency. Although the degree of disintegration attained is lower, there is much closer agreement between the replicates.

On account of the difficulty of interpreting the results of the Sediment Weight Method, it was decided to continue the investigation by Robinson's Method, if significant differences could be obtained.

II. ROBINSON'S METHOD.

The following procedure was adopted for sampling: The pipette, fitted with a large cork and adjusted for depth as described by Robinson, was placed in communication with a filtering flask by means of a length of ordinary connection rubber-tubing. The filter flask was fitted with two small glass tubes; one served for the rubber tube connected with the pipette, the other placed the flask in free communication with the air, so that unless this was closed with the finger, there was no reduction of pressure in the pipette. The side tube of the filter flask was connected

with a filtering pump as usual, but the pump was only allowed to run slowly. About 10 seconds before the appointed time, the rubber tube above the pipette was pinched between the finger and thumb and lowered into position, and the glass tube closed with a finger of the other hand. Five seconds before the appointed time the pressure on the rubber tube was relaxed so that the liquid rose slowly in the pipette. When the pipette was full (this generally took about 10 seconds), the rubber tube was again pinched, and the finger removed from the glass tube. The level was adjusted if necessary, and the sample delivered into the weighed dish. It is not claimed that in this way there was *no* disturbance, but the operation was under better control than could be attained by the mouth.

Triturition with a Rubber Pestle and the Influence of the Rate of Wetting.

A rubber pestle was made by filing down a large rubber bung to the shape of an ordinary pestle, and mounting it on a glass rod which served as a handle. It was found that the removal of the hard vulcanised skin of the bung gave an instrument which was far more efficient in breaking down the hard crumbs than either an ordinary pestle or an untreated rubber bung.

In searching for an explanation of the high degree of disintegration attained in No. 74, it was decided in Series VII to compare the effects of moistening the sample fairly rapidly to a cream with the effects of moistening it very slowly. The resultant suspensions were examined by Robinson's Method, and the results obtained are given in Table VI. It would appear to be established from this experiment that the rate of wetting is a significant factor on the degree of dispersion that will be attained in the preparation of a suspension. It was concluded that this was the probable explanation of the result obtained in No. 74.

Table VI.

	Series VII. Triturated with Rubber Pestle only						Series VIII. Triturated with Rubber Pestle, moistened slowly; then vibrated for 1 hour at 10,000 vibrations per minute							
	Moistened rapidly			Moistened slowly										
Lab. No.	(85)	(86)	Mean	(87)	(88)	Mean	(90)	(91)	(92)	(93)	(94)	(95)	Mean	S.D.
Log $\frac{\text{depth}}{\text{time}}$														
$\bar{1}$·0	81	75	78·0	80	75	77·5	80	91·6	78·8	81·0	82·8	81·2	81·0	1·3
$\bar{2}$·5	60	55		60	61	60·5	58	71·0	65·0	64·0	67·0	66·0	65·0	4·5
$\bar{2}$·0	38	40	39·0	43	45	44·0	52	48·8	48·8	51·0	49·5	51·0	50·0	1·2
$\bar{3}$ 5	32	25	28·5	35	36	35·5	37	37·1	38·0	38·0	(31·0)	41·0	38·0	1·5
$\bar{3}$·0	25	16	20·5	25	27	26·0	29	29·8	31·4	31·4	30·0	37·0	31·0	2·7
$\bar{4}$·5	15	10	12·5	20	19	19·5	?	26·5	26·0	24·0	25·5	(20·8)	25·5	0·9
$\bar{4}$·0	9	8	8·5	11	12	11·5	—	19·1	20·5	21·6	20·6	20·8	20·5	0·8
$\bar{5}$·5	4, 4	3, 6	4·25	8, 11	8, 9	9·0	6	7·4	10·8	8·6	6·9	7·0	7·8	1·6

Triturition with a Rubber Pestle with slow moistening, followed by Vibration.

Although the result obtained in the last experiment was comparable with that given by the beaker method of mechanical analysis, the material was so exceptional that it was considered advisable to test the effect of vibration after triturition. The results obtained from a set of six parallels in Series VIII gave a clay content of 20 per cent. as compared with 11 per cent. with triturition alone. As will be seen on reference to Table VI, the agreement between replicates is satisfactory.

A comparison of various preliminary treatments was then made by Robinson's Method on the Wenlock Shale material, a Lias soil from Kesteven (Great Gonerby Moor), a Coal Measure soil, and a Buttery Clay.

The treatments adopted were as follows:

IX. 2·5 gramme samples were triturated with a rubber pestle, the sodium carbonate solution being added very slowly. The suspensions were then vibrated for one hour, being stirred at intervals during this treatment.

X (a). As in IX, but 10 gramme samples were taken.

X (b). The samples were allowed to stand for one hour in contact with $N/5$ hydrochloric acid as in the English beaker method. They were then transferred to a filter and washed with distilled water until the washings were neutral to litmus paper. The residues were then worked up with the rubber pestle and vibrated for one hour in the sodium carbonate solution.

XI. The samples were moistened very slowly during the vibration treatment, only 10 c.c. being added during the first hour.

XII. *Triturated only.* 20 gramme samples were triturated in a mortar with a rubber pestle, the sodium carbonate solution being added very slowly during this operation· The cream so obtained was gradually diluted and made up to a litre.

XIII. *Triturated in acid.* 20 gramme samples were triturated in $N/5$ hydrochloric acid with a rubber pestle, the acid being added very slowly. After they had been in contact with the acid for an hour, they were thrown on to a filter, and washed with distilled water until the washings were neutral to litmus paper. The residues on the filter paper were then suspended in the sodium carbonate solution without further triturition or prolonged shaking.

XIV. 20 gramme samples were dropped into $N/5$ hydrochloric acid where they were allowed to remain for one hour. They were then washed with distilled water until the washings were free from acid as before. The residues on the filter paper were then carefully triturated with a rubber pestle, the sodium carbonate solution being added very slowly, and were finally made up to a litre.

An examination of the results obtained (Table VII and Figs. 3—6) shows that treatment VIII or IX gives the highest degree of disintegration in each of the four soils examined, the increase being most marked in the case of the rather exceptional Wenlock Shale material. The increased dispersion resulting from vibration after triturition as compared with triturition alone, revealed by a comparison of IX and XII, is very great in the Wenlock Shale and in the Lias, but is not shown in the other soils to such a great extent.

Table VII.

Lias.

Log $\dfrac{\text{depth}}{\text{time}}$	IX	X (a)	X (b)	XI	XII	XIII	XIV
$\bar{1}\cdot0$	65·9	68·8	65·8	63·5	62·5	59·5	66·3
$\bar{2}\cdot5$	60·3	65·0	63·0	55·6	58·5	56·8	63·0
2·0	66·7	61·0	57·5	48·7	50·0	49·8	58·0
$\bar{3}\cdot5$	51·7	52·3	51·8	40·7	37·8	45·8	49·8
3·0	46·0	49·3	44·3	33·3	34·3	42·3	43·8
$\bar{4}\cdot5$	44·0	44·0	43·0	24·2	28·3	40·0	43·5
4·0	39·7	37·5	38·0	14·8	19·75	36·8	38·3

Coal Measures.

Log $\dfrac{\text{depth}}{\text{time}}$	IX	X (a)	X (b)	XI	XII	XIII	XIV
1·0	37·8	28·5	28·8	32·2	28·8	30·5	32·5
$\bar{2}\cdot5$	29·4	28·5	25·3	24·5	23·8	21·3	25·5
$\bar{2}\cdot0$	24·0	18·5	19·8	17·6	18·8	16·8	16·3
$\bar{3}\cdot5$	21·0	16·0	17·0	14·8	17·5	16·8	16·3
$\bar{3}\cdot0$	18·0	10·8	16·3	?	16·3	14·5	13·0
$\bar{4}\cdot5$	16·8	9·8	13·8	8·8	13·8	13·3	11·8
$\bar{4}\cdot0$	14·4	9·0	11·0	4·7	11·3	6·3	10·8

Buttery Clay.

Log $\dfrac{\text{depth}}{\text{time}}$	IX	X (a)	X (b)	XI	XII	XIII	XIV
$\bar{1}\cdot0$	66·4	75·3	61·5	61·3	67·5	58·5	62·5
$\bar{2}\cdot5$	41·7	56·5	47·0	44·3	54·8	46·3	42·0
$\bar{2}\cdot0$	40·8	40·8	35·3	33·5	38·5	34·0	31·3
$\bar{3}\cdot5$	32·9	36·0	27·3	28·6	33·0	27·5	24·3
$\bar{3}\cdot0$	28·8	30·8	21·8	23·1	28·5	24·3	20·5
$\bar{4}\cdot5$	24·1	24·8	15·5	13·2	24·3	18·0	12·8
$\bar{4}\cdot0$	20·0	20·5	11·3	12·4	20·8	15·3	12·8

Wenlock Shale Material.

Log $\dfrac{\text{depth}}{\text{time}}$	VII (a)	VII (b)	VIII	XI	XII	XIII	XIV
$\bar{1}\cdot0$	78·0	77·5	81·0	62·0	90·5	74·3	76·3
$\bar{2}\cdot5$	58·0	60·5	65·0	50·0	64·3	56·3	56·3
$\bar{2}\cdot0$	39·0	44·0	50·0	35·2	44·0	41·8	40·0
$\bar{3}\cdot5$	28·5	35·5	38·0	24·8	34·3	28·5	24·3
$\bar{3}\cdot0$	20·5	26·5	31·0	17·4	25·3	21·0	17·8
$\bar{4}\cdot5$	12·5	19·5	25·5	12·4	16·8	16·0	12·3
$\bar{4}\cdot0$	8·5	11·5	20·5	7·0	11·8	9·5	7·0

The addition of acid treatment was apparently without effect on the Lias, but caused an undoubted reduction in the content of finer fractions in the Buttery Clay. The result in the case of the Coal Measure is rather contradictory. Acid apparently halves the clay content in the non-vibrated set (comparing XII and XIII), but this effect is destroyed if the acid treatment is followed by triturition in soda (XIV). On the other
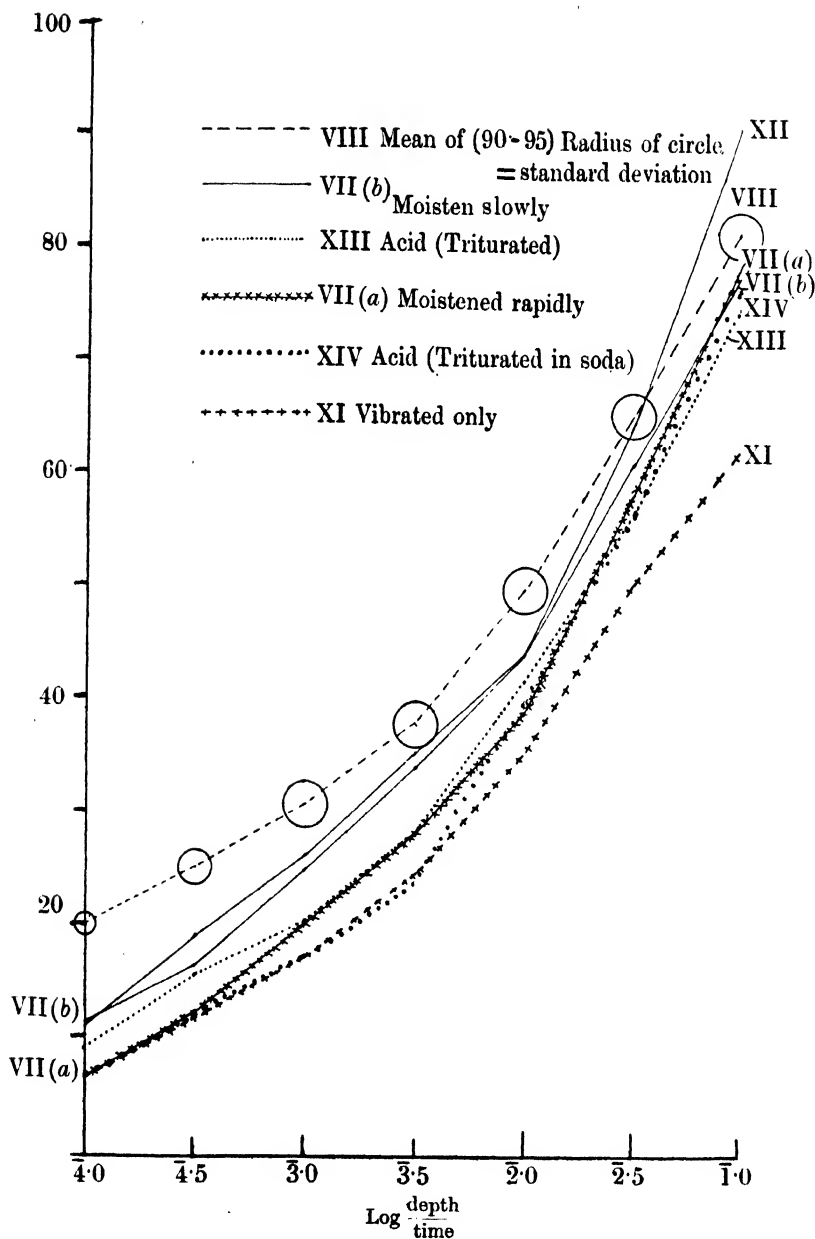
Fig. 3. Wenlock Shale
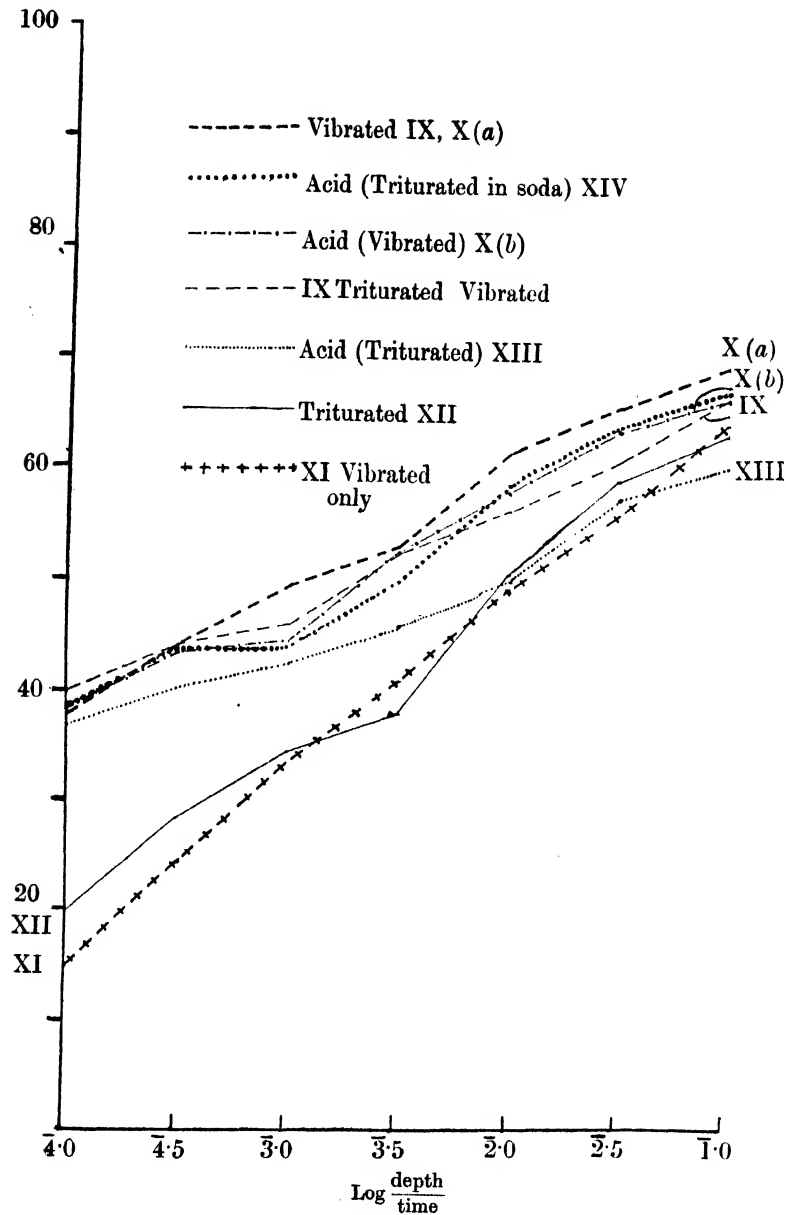
Fig. 4. Lias
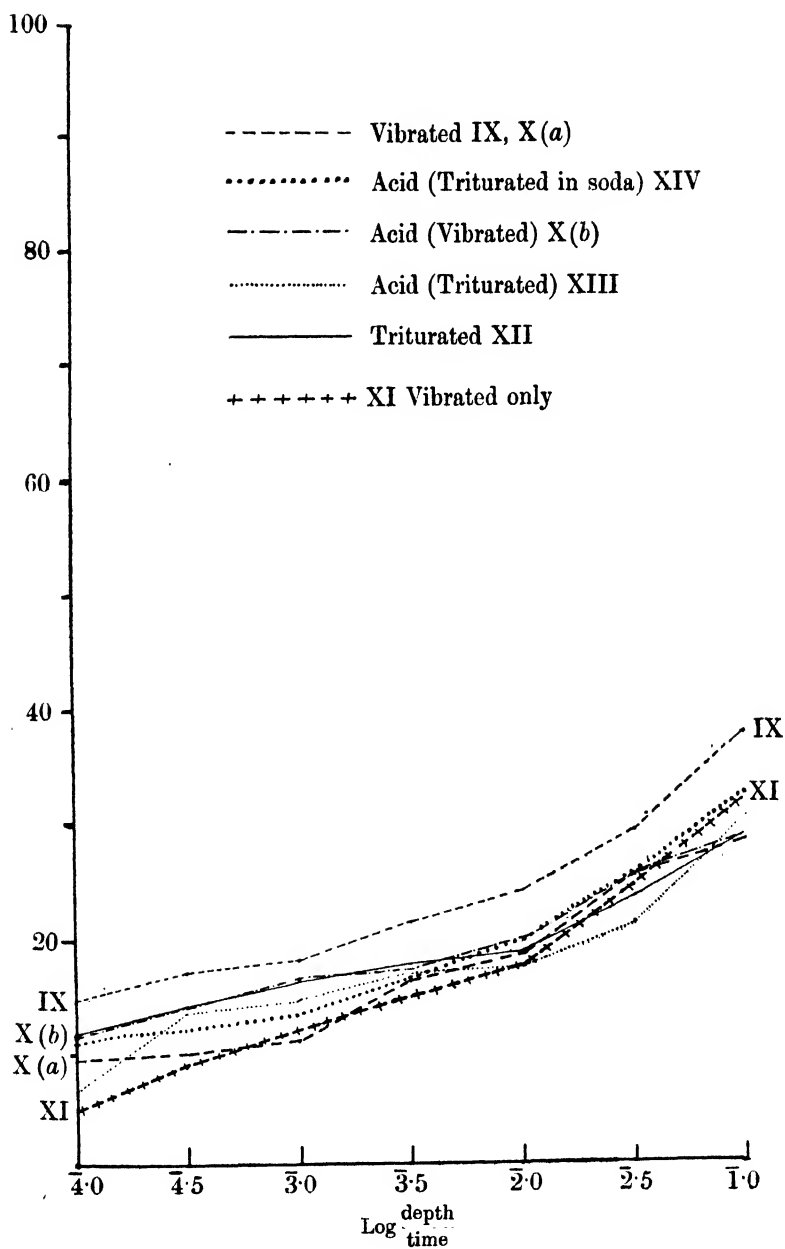
Fig. 5. Coal Measure
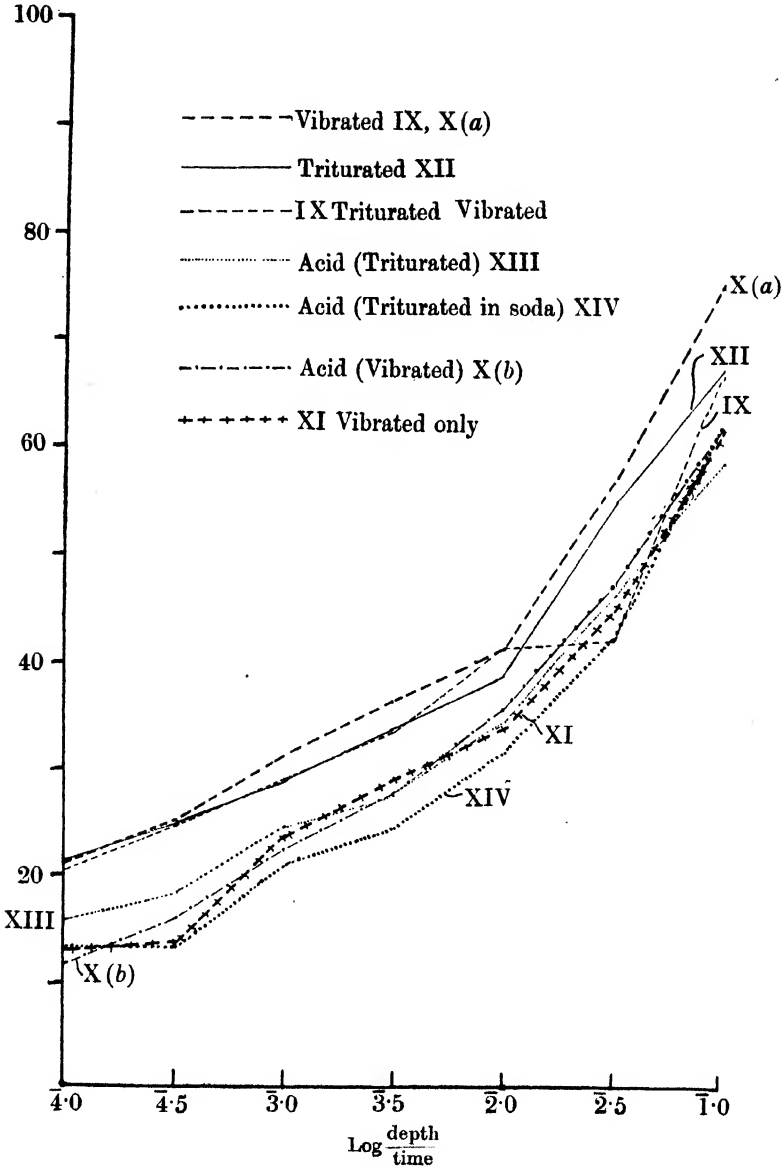
Fig. 6. Buttery Clay

hand, the clay content is still below that given in IX, with which X (a) does not agree very well. It is thought that the higher concentration employed in X (a) may have caused a certain amount of flocculation. The duplicates in IX showed good agreement.

It was thought that by combining slow moistening with vibration, it might be possible to obtain a satisfactory degree of dispersion without triturition. This would have had the great advantage of eliminating the personal error involved in triturition. Treatment XI shows clearly that triturition cannot be dispensed with in the case of heavy soils.

Triturition alone without vibration (XII) appears to be satisfactory in the case of the Buttery Clay and also possibly in the case of the Coal Measure soil, but fails with the Lias.

General Discussion.

The line of reasoning which led to the elaboration of the method of Series VIII and IX has been given in detail in the description of the experimental work and need not be repeated here. There are, however, a few points which deserve further consideration.

The problem of frequency of vibration still requires a great deal of investigation. In the only experiments which have been reported here in this connection (Series I and II) the material consisted initially of uniformly large particles, which, as they were gradually disintegrated, must speedily have given rise to a mixture of particles of all sizes, the larger ones still predominating.

It would appear to be probable that a different range of frequency would be most effective in breaking up aggregates of different sizes, and from the results obtained with hand shaking it would appear that with large aggregates a low frequency is more effective than a high one. This point was examined qualitatively in the following way. Some of the hard resistant aggregates which remained on the 100 mesh sieve in the ordinary beaker method of mechanical analysis were placed in the vibrating vessel with water, and vibrated. The frequency was varied throughout the available range, *i.e.* from 2000 to 2750, from 4000 to 5500, and from 8000 to 11,000 vibrations per minute, the behaviour of the particles being observed very carefully at all frequencies. It was found that the cloud of fine particles which ascended from the dancing aggregates was certainly more voluminous and darker in colour at the higher frequencies. As the governor of the gramophone motor did not admit of more than 11,000 vibrations per minute, the investigation of still higher frequencies has been postponed for the present.

A comparison of the results obtained with the different treatments leads to the conclusion that triturition in a mortar with.a rubber pestle, the soil being moistened very slowly, followed by one hour's vibration at 10,000 vibrations per minute with occasional stirring of the suspension during this operation, gives a degree of disintegration that would appear to be fairly satisfactory. It is quite possible that with ordinary undried soils the period of vibration could be considerably reduced. Further, it would appear to be established that the use of dilute acids is not advisable, since in three out of the four soils examined it has caused an apparent reduction of the finer fractions, while in the fourth case, the Lias, there is certainly no increase. The reduction is most marked in the Buttery Clay, where with 7 per cent. calcium carbonate an increase would have been expected. The author is of the opinion that this reduction of the finer fractions is due to flocculation and aggregation by the acid, and is not as a rule due to a solvent action. The fact that triturating the acid treated residue with dilute sodium carbonate solution caused a slight increase of the finer fractions as compared with immediate suspension supports this view. Attention has already been called to the work of Hissink who does not stress this point, although the evidence is provided.

The necessity for moistening a soil slowly in order to obtain a satisfactory suspension has already been noticed by American bacteriologists, but the point does not appear to have been brought forward in connection with mechanical analysis. The manufacturers of various patent foods always advise that the liquid be added slowly, and this practice is regularly adopted in connection with other culinary operations. It is suggested that the real object is to ensure that each individual grain of the dry material comes into contact with the liquid so that it is actually wetted by the liquid. For instance, oatmeal is slowly sifted into water in order to prevent the formation of lumps. With rapid additions of either ingredient, aggregates are formed which are wet on the exterior but have a dry centre. If a sample of a moist clay soil be shaken violently with dilute sodium carbonate solution in a flask for a short time, the liquid becomes turbid, but on pouring off the turbid liquid, a large proportion of the clay is found to have been moulded into spheres. These are coated with a wet layer of puddled clay which effectively protects the inner portion from coming in contact with the liquid. With gradual moistening and triturition these aggregates are extremely small and are comparatively easily disintegrated by vibration. If a long kneading process is adopted it is possible that there may be no necessity for vibration. The Buttery Clay result (XII) illustrates this.

On boiling a small quantity of soil in a beaker containing water, it is found that the soil particles dance on the bottom of the beaker in much the same way as they do on the membrane of the vibrating vessel. It is suggested that vibration possesses all the advantages of boiling without any of the disadvantages due to the high temperature and chemical action, accompanied by coagulation of the clay fraction.

SUMMARY.

1. An apparatus is described which has been designed to impart a known number of vibrations per minute to a suspension of soil or other material.

2. The problem of the calibration of the apparatus is briefly discussed.

3. A new method for the comparison of the mechanical composition of suspensions is described.

4. Various preliminary treatments of samples of soil for mechanical analysis are compared, and it is shown that:

(a) The rate of wetting is an important factor.

(b) A combination of triturition and vibration gives a satisfactory degree of dispersion.

(c) The use of acids is not advisable.

APPENDIX.

Mechanical Analyses by the Beaker Method.

	Wenlock Shale	Lias	Buttery Clay
Fine gravel ...	0·0	0·53	0·0
Coarse sand ...	0·0	10·55	0·0
Fine sand ...	10·65	6·87	19·55
Silt	31·41	8·10	24·38
Fine silt ...	34·95	16·89	14·65
Clay	10·76	41·73	21·88
Calcium carbonate	—	·22	6·95

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A PRELIMINARY INVESTIGATION INTO THE DRAFT OF THE PLOUGH.

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(With Fifteen Text-figures.)

INTRODUCTION.

FOR some considerable time agriculturists have felt the desirability of enquiring more closely into the draft of various farm implements and the necessity of making accurate draft measurements under normal working conditions.

It is customary among farmers to refer to a particular soil as being a "two-horse soil" or a "three-horse soil," as the case may be, meaning thereby, that two horses or three horses respectively are needed to draw a plough in that soil, cutting a furrow slice of average dimensions.

The object in starting this work was, therefore, to attempt to discover the magnitude of the draft of a plough working firstly, under normal conditions, and secondly under varying conditions. For this purpose it was necessary to construct a self-recording dynamometer, which would register graphically changes in draft over a given distance.

One of the earliest series of draft measurements recorded was made by Pusey[1] in 1840. By means of a simple dynamometer he was able to obtain average readings which enabled him to compare the relative drafts of ten different kinds of ploughs. Later, during the last two decades of the nineteenth century, an extended series of investigations was carried out by Professor J. W. Sanborn[2] in America, not on ploughs only, but on many kinds of farm implements when working under various conditions.

Quite recently, Professor Davidson (Iowa State University) has carried out experiments showing the relation between the draft of a plough and the speed of traction.

The reports of the Tractor Trials held in 1919, 1920 and 1921 also

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[1] *Journal of the Royal Agricultural Society of England,* 1840.
[2] King, *Physics of Agriculture.*

furnish useful data, relating to the draft of multiple furrow ploughs used in conjunction with various makes of tractors. Nevertheless very few figures are available in this country which throw light on the effect of the many factors which influence draft.

It is evident that there are two lines of investigation open, either to discover the pattern of plough working with least draft under uniform conditions, which should be a maker's problem rather than an agriculturist's, or to investigate the influence of varying working conditions on one particular plough. It was decided to pursue the latter course, and a plough was chosen which was thought suitable for use under all conditions; the implement was a Howard General Purpose Single Furrow plough weighing 276 lbs.

At the outset the coulter was freshly dressed, a new B 2 pattern share fitted and the plough was used sufficiently to polish all parts in frictional contact with the soil.

The same pair of horses was used throughout (except in Series A and B), chosen as being likely to pull evenly at a good pace and unlikely to bolt or be worried by the continual stopping and starting which necessarily had to take place.

The actual working necessitated the presence of two operators, one to handle the plough and guide the horses (this work was usually performed by the author's brother) and the other to attend to the instrument and see that it was recording satisfactorily.

The work was begun on the Cambridge University Farm in the summer of 1920, five preliminary measurements being made to get the apparatus in proper working order and to determine in what way the other measurements (such as depth of furrow) should best be made.

All the remaining measurements were made on the Folley Farm, Lydbury North, Shropshire in the winter 1920–1921.

THE APPARATUS

The type of apparatus was self-recording and similar to that used by Landon in measuring the draw-bar pull of steam tractors, and giving a force/distance chart.

The dynamometer consists of a helical spring mounted in an iron cradle, one end of which is connected through the whipple trees to the horses, and the other to the draft chain of the plough. When a pull is exerted by the horses the spring is contracted. The contraction is communicated by means of a Bowden wire to a pencil moving over a paper fixed on a revolving brass drum.

Bowden wire

18½"

32½"

25"

Spring ¼" Matl. 1" Pitch

6¾"

¼" Plate

Bar ¾" Screwed

Scale   full size.

8" Flywheel
rim ½"

4½"

12"

4½"

12"

3"

Spring

¼" rod

10¼" long

Bowden wire

Wheel 18" diam.

Trailing wheel. Scale   full size.
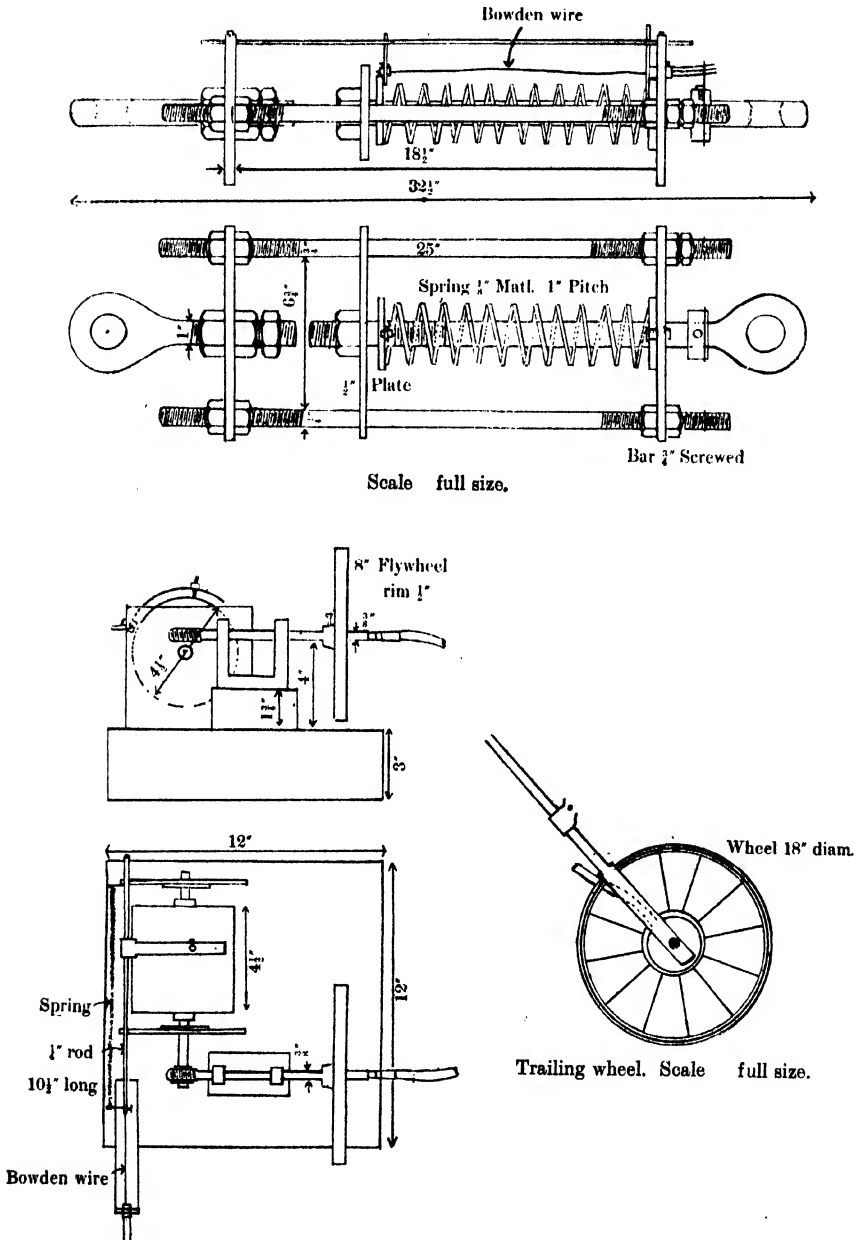
Fig. 1.

The drum is mounted on a small wooden base bolted to an iron upright, which in turn is fixed to the beam of the plough near the point of attachment of the foot. Rotation is imparted to the drum by means of a flexible connection driven by gearing from a wheel trailing in the furrow. The drum is rotated once in every 26 yards and owing to this slow motion it was found necessary to attach a fairly heavy fly-wheel to the driving spindle to impart an even motion to the drum, which otherwise rotated with a jerky motion.

The trailing wheel attachment is clamped to the plough stilts. Between the wheel and the clamp, a union is provided, which allows
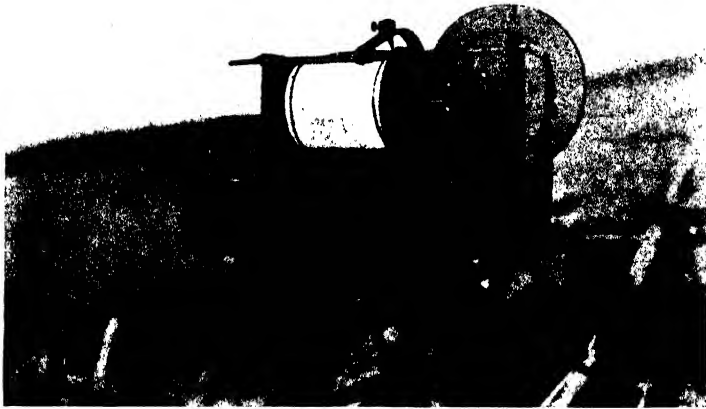
Fig. 1a.

the wheel free vertical movement, enabling it to adapt itself to varying depths of furrow and to temporary tilt of the plough caused by obstacles. The position of the wheel is just under the handles of the plough and sufficiently forward to allow the ploughman freedom in guiding the plough.

Fig. 1 is a scale drawing of the apparatus showing the dimensions and the relative position of the parts when working.

Fig. 1a is a photograph showing the drum with its cable attachment from the trailing wheel at the back, and the Bowden wire actuating the pencil from the large spring at the front. The position of the fly-wheel is also shown and the gearing rotating the drum. It will be seen that the

Bowden wire is attached by a small screw to a brass rod on which the pencil holder moves. The holder can be clamped at any desired point, and in this way a suitable zero line on the paper can always be chosen before making a measurement. The small spring by the side of the brass rod takes up any slackness in the Bowden wire.

The total weight of all parts of the dynamometer is 114 lbs.

CALIBRATION OF THE DYNAMOMETER SPRING.

The spring was removed from its cradle and placed in a Buckton Testing Machine with the Bowden wire connecting it to the recording pencil attached. In this way, when a definite pressure was exerted by the machine it was possible to measure the contraction of the spring directly on the millimetre squared paper fastened to the drum. A series of readings was taken at different pressures and a curve (Fig. 2) obtained showing the relation between the pressure exerted in lbs. (ordinates) and the contraction of the spring in millimetres (abscissae). From this curve the mean draft in pounds of every chart was obtained.

Fig. 2. Relation between compression of spring in millimetres and draft in lbs.

TYPES OF SOIL ON WHICH THE INVESTIGATIONS WERE MADE.

The measurements included in Series A were made on a level field on the Cambridge University Farm, the texture being of a gravelly nature of medium tenacity.

The remainder of the tests were carried out at the Folley Farm on soil overlying shales of the Wenlock Formation, and the mechanical analyses, as shown in the tables, indicate considerable uniformity over all the farm. The type of soil may be described as a Medium Loam, due to the presence of a high silt content, both fine and coarse, combined with a rather low content of sand and clay.

A list of the fields in which the measurements of draft were made is given in Table I. Opposite the name of the field will be found the Ordnance Survey Number, the gradient at the point where the measurements were made, and the index letter of the series of measurements taken in the field.

Table I.

Name of field	Ordnance Survey No.	Gradient	Series
Fields B and C (University Farm, Cambridge)	—	Level	A
Cherry Tree Piece (Folley Farm)	621	1 in 8·2	B
Fifteen Acres ,,	622	1 in 9·2	C and D
Orchard field ,,	600	1 in 8·2	E
Chestnut Piece ,,	603	1 in 7·2	F
Plum Piece ,,	602	1 in 8·3	G
Five Turnings field ,,	638	1 in 17	H
Twelve Acres ,,	569	Level	I and J
Yew Tree Piece ,,	585	Level	K and L

MAKING MEASUREMENTS IN THE FIELD.

A site was first chosen suitable for obtaining a series of measurements. If the field was level, as in Series A, I, J, K, L, this was simple and measurements could be made anywhere, but if the field sloped, then a portion of land was chosen where the slope was uniform for a distance at least 100 feet in length. As most of the fields sloped, a set of measurements had to be made both uphill and downhill in order to get an average value.

In general, a piece of land where furrows had already been ploughed out was chosen; if this could not be obtained, a number of furrows had to be ploughed before starting, to facilitate turning at the ends and to obtain a furrow for the horse to walk in.

If land was allowed to remain partially ploughed for some time the rectangular edge of the unploughed land adjoining the furrow became broken and dried, so in any case it was necessary to plough once round before starting to make measurements. The land having been prepared, the apparatus was fitted to the plough and well oiled and the plough adjusted to the approximate depth and width required. The horses were hitched, two abreast, and the pencil fixed on the zero line chosen. It was the duty of the ploughman to hold the furrow wheel well up to the edge of the unploughed land without allowing the wheel to cut into it.

Having obtained a reading it was usual to plough round the land and make another measurement on the opposite side. Thus a series of readings was made consisting of one set when the plough travelled uphill and the other when the plough travelled downhill.

deep ploughing

Zero

med depth

Zero

shallow

Zero

direction of ploughing

III c.

III b.

III a.
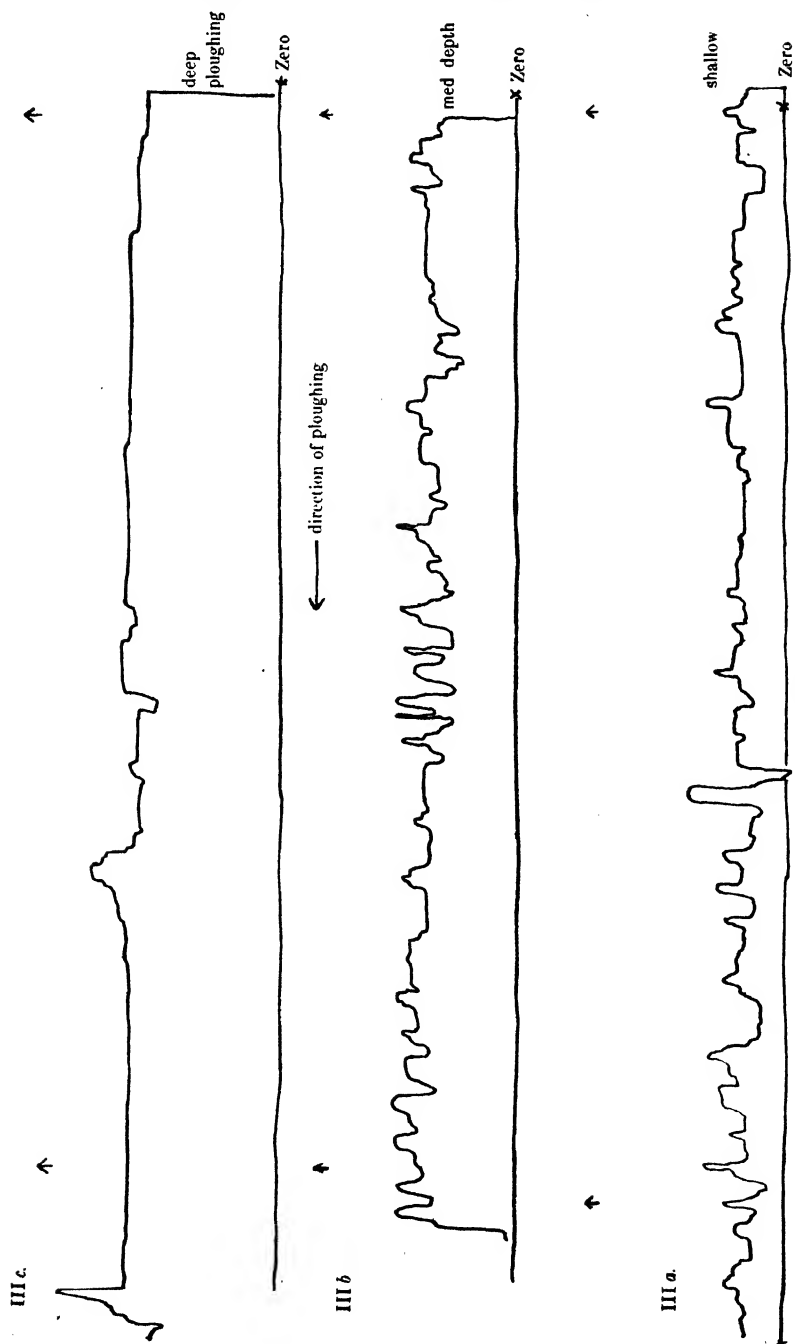
Fig. 3.

Specimens of charts obtained in the field are shown in Fig. 3 (*a*, *b* and *c*).

The direction of ploughing, indicated by the horizontal arrow, was over a distance of approximately 45 yards.

Fig. 3 *a* represents the type of graph obtained when the furrow is shallow and shows considerable unevenness.

Fig. 3 *b* shows the type of graph obtained when ploughing at usual depths. It will be noticed that the line is less uneven than in Fig. 4 *a*, due no doubt to the steadying effect of a greater weight of earth passing over the share and breast.

Fig. 3 *c* shows the type of graph obtained in deep furrows. This line is particularly steady, due, no doubt, to the considerable weight of earth passing over the breast, thus minimising the jerky pull of the horses and the momentary tendency of obstacles to increase the draft.

In one case (Series I) ploughing deep where the soil was rather shallow, a particularly uneven graph was obtained. This was the result of the share cutting into the rock below.

MEASUREMENT OF DEPTH AND WIDTH OF FURROW.

Depth. In taking readings of the depth the method adopted was as follows. A board about 2 ft. 6 ins. long was laid flat on the unploughed ground with about 6 ins. projecting over the furrow. Pressure was applied to the board to flatten any small lumps of earth and bring its surface into close contact with the surface of the earth. A ruler was held perpendicular to the board touching the bottom of the furrow about half way across the furrow, and a reading taken. It was found that the depth of the furrow varied very considerably, even when measurements were made only one foot apart, so it became necessary to take at least ten readings over the distance ploughed in order to obtain a fair average.

Width. The width was measured by the distance from the coulter, just as it was beginning to cut the soil, to the edge of the furrow. In this case it was necessary to stop the plough while making measurements. Three or four measurements were usually taken to obtain a mean value.

SOIL SAMPLES.

Soil samples were taken in every case for the purpose of ascertaining the mechanical composition and the moisture content of the soil at the time of the experiment.

The method adopted for sampling was to take several cores at an average depth of 5 ins. in the unploughed land adjoining a set of furrows

in which draft had been measured. These cores were well mixed and a sample placed in an air-tight tin for analysis. Since two sets of measurements were made in the sloping fields, two soil samples were usually taken, one sample representative of an uphill set of measurements and the other sample of the downhill set of measurements. The average figure of these two analyses was used when tabulating the results. Where draft measurements were made in a level field only one soil sample was taken.

The various constituents are expressed in parts per hundred of air-,dried soil, except the moisture, which is expressed as a percentage calculated on 100 parts of air-dried soil.

METHOD OF CALCULATING THE MEAN DRAFT OF A CHART.

An approximate mean is chosen. Then, in order to ascertain the necessary correction, the squares on the chart included between the selected line and the graph, above and below this, are counted separately. The difference between the sums is divided by the total length of the curve over which the count has been made (denoted by the distance between the vertical arrows), thus obtaining the correction. The true mean is then calculated in millimetres.

Referring this value of the true mean to the curve in Fig. 2, the corresponding draft in lbs. is obtained. For example, in Chart No. 50, the draft expressed in millimetres is 19·21. By referring this number to the abscissa in Fig. 2, the corresponding ordinate value of 180 is obtained, which represents the draft in pounds.

MEANING OF "DRAFT."

The mean draft as calculated from one of the charts is a measure of the force required to keep the plough moving uniformly through the ground at a pace of 2 to $2\frac{1}{2}$ miles per hour. This force is used to perform two operations, one to produce movement in the plough, and the other to cut and turn the furrow slice. A distinction, therefore, has to be made between the "Gross Draft" required to move the plough as well as to cut and turn the furrow slice, and the "Nett Draft" which is the force required to cut and turn the furrow slice only.

The manufacturer is more interested in the gross draft which should be reduced to a minimum for any given work, while the agriculturist is more interested in the nett draft which is a guide to the relation between cultivation and the soil conditions.

CALCULATION OF THE NETT DRAFT FROM THE GROSS DRAFT.

The deadweight draft of the plough and apparatus, running free, was found to be 87 lbs. So, in the case of a level field the gross draft, less 87 lbs. gives the nett draft. But in the case of a sloping field it becomes necessary to calculate a correction for the gradient, which must also be deducted from gross draft when ploughing uphill, and added when ploughing downhill. Knowing the total weight of the plough and apparatus (390 lbs.) and the inclination of the ground (given in Table I), this is done by applying the principle of work and the inclined plane.

In Series B, the inclination of the field at the point where the measurements were made is 1 in 8·2, increasing the draft uphill by 48 lbs.; therefore, from every gross draft uphill subtract (87 + 48) lbs., to obtain the corresponding nett draft. Similarly from every gross draft downhill subtract (87 − 48) lbs. to obtain the nett draft.

DRAFT PER SQUARE INCH OF FURROW SLICE.

If the draft be divided by the area of cross section of the furrow slice, a value is obtained which expresses the resistance offered by each square inch of furrow slice. If the gross draft (in lbs.) be divided by the area of cross section in sq. inches, a value Δ is obtained, and if the nett draft be divided a value δ is obtained.

Such a course has been followed by previous investigators and for that reason has been followed here. It does not claim to be an adequate expression of draft. Probably some other expression would serve equally well or even better.

Actual measurements show variation in the value of δ and suggest that it is not entirely independent of both width and depth. Instances of these variations and their probable meaning will be discussed under each series of measurements.

THE FACTORS AND CONDITIONS INFLUENCING DRAFT.

It has already been stated in a previous part of this paper that draft is subject to the influence of numerous conditions, some relating to the plough and working conditions, which are included in the following first list, and others relating to the soil, included in the second list.

A more detailed discussion of the effect of some of the conditions will be found later in the paper.

I. *Conditions relating to the plough.* (*Mechanical conditions.*)

(*a*) Weight of the plough.

(*b*) Pattern of mould board or breast (*i.e.* digger or general purpose shape).

(*c*) Pattern of share.

(*d*) Variety of coulter.

(*e*) Sharpness of coulter and share.

(*f*) Position and line of hitch.

(*g*) Rate of ploughing.

(*h*) Inclination of the ground.

II. *Conditions relating to the soil.*

(*a*) Texture (expressed by mechanical analysis).

(*b*) Degree of consolidation:

(1) at different depths; (2) following different crops.

(*c*) Moisture content.

(*d*) Width and depth of the furrow.

(*e*) Presence of obstacles.

So far the experiments have been confined to the investigation of the influence on draft of varying conditions in the soil rather than to the effect of variation of the mechanical conditions.

When investigating the effect produced by the variation of any one factor, attempts were made to keep all the other factors constant. This frequently proved difficult as is instanced when endeavouring to maintain an even width; whether or not this can be done depends entirely on the care exercised by the ploughman in keeping the large furrow wheel well up to the edge of the unploughed land, without actually cutting into it. Or again there is the difficulty in keeping an even pace throughout, particularly where some measurements are taken uphill and others downhill. The horses used moved more slowly uphill than down, and therefore introduced variations in draft due to speed.

By using the same plough throughout, with the same pair of horses, the various factors included in List I were kept fairly constant. An exception to this was factor I (*h*) (the inclination of the ground) for which a correction had to be introduced. The degree to which the various factors included in List II can be kept constant depends to some extent on the rapidity with which the measurements are made; it would be unwise, for instance, while measuring effect of variations in depth on draft to make some measurements one day and some later, as the moisture content and other factors would probably change during that time.

Of the conditions mentioned, some are much more important than others in their effect on draft. For instance, the effect of obstacles, or the relative degree of consolidation of the ground at different depths (in this particular variety of soil) is probably small, compared with the effect of variations in moisture, depth, and width, and as far as these experiments go they suggest that the former factors are not of primary importance.

Detailed List of Draft Measurements.

The first group, comprising the series B, E, F, G, H, I, K and L, shows the relation between draft and depth of furrow.

The second group, consisting of three series, C, D and J, shows the relation between draft and width of furrow.

A comparison of the mean value of δ in each series gives a good idea of the state of consolidation produced by the previous crop.

In presenting the results, wherever two sets of measurement were necessary in a field, they have been grouped together in one series. Otherwise, where the field was flat the series consists of one set of readings only.

Series A.

Table II. Preliminary measurements made on University Farm to get apparatus in working order.

Nos. 1, 2, 3, 4. Field B. Ploughing after folded catch crop.
No. 5   Field C. Ploughing loose surface soil.
May 18th, 1920.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Moisture %	Mechanical analysis %
1	10	4·63	478	10·3	391	8·45		⎰ 11·3 stones and gravel
2	10	4·63	470	10·1	383	8·30	Not recorded	{ 38·5 sand
3	10	4·63	397	8·6	310	6·70		{ 16·5 silt
4	10	5·88	480	8·2	393	6·69		⎱ 13·3 clay
		June 1st, 1920.						⎰ 11·2 stones and gravel
								{ 48·9 sand
5	10	4·5	355	7·88	268	5·95		{ 17·2 silt
								⎱ 10·9 clay
76	—	—	87	—	—	—	Plough and apparatus running empty

Chart No. 1. Surface of the land had been hardened by folding sheep on it, and the ground hard under the surface.

Chart No. 2. Made under similar conditions to No. 1, but the sheep had been folded for a shorter period.

Chart No. 3. Made under similar conditions to No. 2, but in a different part of the field.

Chart No. 4. Similar to No. 3, but ground appeared stiffer and depth also greater.

Chart No. 5. Ploughing in a cultivated soil, with a loose surface.

Really each separate chart is a series in itself inasmuch as it gives a figure for a particular set of conditions.

Chart No. 76 shows the draft of the plough and apparatus running empty on a level field. From this figure all the nett draft corrections have been made.

Inspection of Table II shows the number of the chart in the first column, followed by width and depth in the second and third columns, and gross draft in the fourth column; this shows a variation from 355 lbs. to 480 lbs. Δ is shown in the fifth column and expresses the gross draft per square inch of furrow slice.

The nett draft is shown in the sixth column and shows a variation from 268 lbs. to 393 lbs.

The remaining figures show the various mechanical constituents of the soil.

In the following groups it will be noticed that each series is denoted by a capital letter. The alphabetical sequence of these places the series in the order in which they were taken. But for purposes of arrangement into groups that order has been altered, the series, however, keeping their original index letter.

First Group. Variation of draft with depth of furrow consisting of Series B, E, F, G, H, I, K, L.

<div align="center">

SERIES B.

Table III. Variation of draft with depth.

Cherry Tree Piece. Ploughing two year old clover ley.

Gradient 1 in 8·2. Gradient correction ± 48 lbs.

October 18th, 1920.

</div>

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis
Uphill							
8	10	3·4	238	7·0	103	3·03	
10	10	4·6	355	7·75	220	4·79	
12	10	6·1	470	7·7	335	5·49	
14	10	7·4	550	7·46	415	5·60	25·7 moisture
			Δ av. = 7·48		δ av. = 4·73		31·2 stones and gravel 12·6 sand
Downhill							48·5 silt
9	10	3·7	107	2·88	68	1·84	12·4 clay
11	10	5·2	214	4·08	175	3·34	
13	10	7·0	414	5·9	375	5·35	
15	10	8·4	450	5·35	411	4·9	
			Δ av. = 4·55		δ av. = 3·86		

<div align="center">

Average for the series: Δ = 6·0; δ = 4·30.

</div>

The series B consists of two sets of measurements made on a two year old clover ley during fine weather, when the soil was moist from recent rains. This was the first series of measurements made on a sloping field. Five measurements were made uphill and five downhill. Column 3 shows depths varying from 3·4–8·4 ins. Actually it is not easy to take accurate measurements at a depth of less than 3·4 ins., because it is impossible to maintain uniform depth.

The process of regulating the depth of a plough to differences of anything less than 1 inch is difficult, so that about five measurements only, at different depths were possible.

The measurements were all taken near the middle of the field, the uphill furrows being adjacent and the downhill furrows adjacent, the two sets being separated by only 4 or 5 yards.

The gradient of the field at the point where the measurements were made was 1 in 8·2, necessitating a correction of 48 lbs. when calculating the nett draft.

VARIATIONS IN DRAFT.

It will be seen that variations from 107 to 550 lbs. took place in the gross draft, and from 68 to 415 lbs. in the nett draft.

VARIATIONS IN VALUES OF Δ AND δ.

Uphill. It will be seen that Δ is nearly constant in value, about 7·5.

The deadweight draft of the plough forms a large proportion of the gross draft at small depths and becomes a relatively smaller part as the depth increases. Therefore at greater depths, Δ and δ would be expected to approach near one another in value. This apparently is the case as Δ is fairly constant in value ($= 7·48$) throughout, while δ shows a steady increase from 3·03 to 5·6.

This increase in the value of δ as the depth increases may be explained by the fact that the ground is more consolidated at greater depths and therefore requires an increased draft per square inch to cut the furrow slice. Also it has been noticed when ploughing a shallow furrow, that the furrow slice is turned easily into its place, but when the furrow is deep, there is considerable back pressure on the breast, caused by the thick slice pressing against the next one. This would suggest that there may be increased work per square inch of furrow slice both in cutting and turning at greater depths. This statement is confirmed by the figures obtained in some of the other series.

Downhill. Considerable diversity in the values of Δ and δ are seen, No. 9 being rather low.

A further discussion of this variability in value of Δ and δ is made under the heading "General discussion," where the data collected from the various series are reviewed.

In Fig. 4 the results of the measurements given in Table III are expressed graphically.
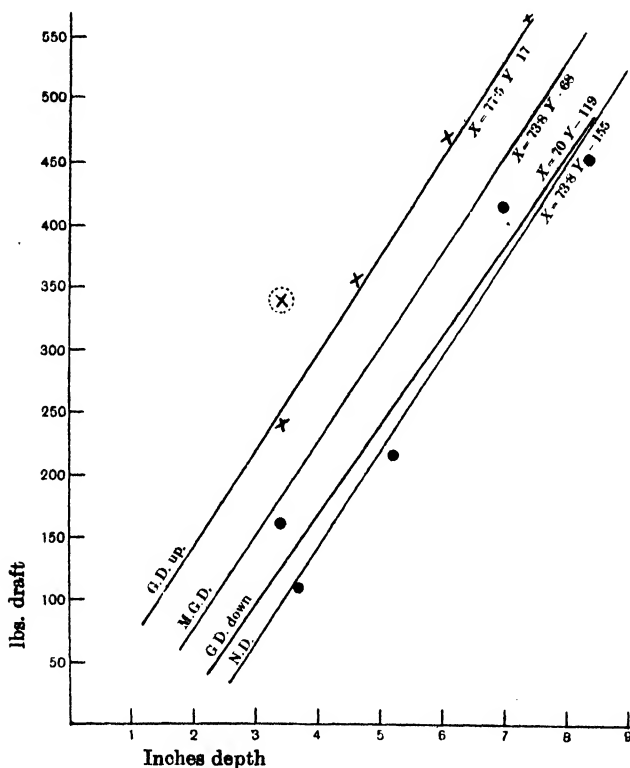
Fig. 4. Series B.

Four curves are shown, three of which show the relation of the gross draft with variation in depth, and the fourth, the relation of nett draft and depth.

The curve of gross draft uphill (G.D. up) is obtained by taking a mean of the points (denoted by crosses) whose coordinates are depth of furrow and the corresponding draft, when ploughing uphill. The exact position of the mean is found by plotting the equation $Y = 77 \cdot 5\, X - 17$. This equation is calculated from the data in the third and fourth columns of Table III by using the method of least squares.

The curve of gross draft downhill (G.D. down) is similarly obtained by plotting the equation $Y = 70\,X - 119$, and is the mean of the downhill points (denoted by dots).

The mean curve of gross draft (M.G.D.) represents the curve which would be obtained if the field were level. Its position is found by plotting the average of the two foregoing equations ($Y = 73 \cdot 8\,X - 68$).

The curve of nett draft (N.D.) showing the relation between the nett draft and depth of furrow is obtained by plotting the (M.G.D.) equation corrected for the dead weight of the plough and apparatus.

$$Y = 73 \cdot 8\,X - 68 - 87 = 73 \cdot 8\,X - 115.$$

It will be observed that the points (denoted by crosses) around the curve G.D. up lie closely to their mean.

The curves point to a linear relation between draft and depth.

SERIES E.

Table IV. Variation of draft with depth.

Orchard Field. Ploughing stubble, farm-yard manure on surface.
Gradient 1 in 8·2. Gradient correction ±48 lbs.

February 21st, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis %
Uphill							
34	9·7	3·7	337	9·35	202	5·62	
36	9·0	4·6	365	8·95	230	5·61	
38	9·8	5·3	395	7·58	260	5·00	
40	9·8	5·7	468	8·40	333	5·95	
41	9·8	6·7	529	8·01	394	5·96	25·5 water
				Δ av. = 8·46		δ av. = 5·63	24·1 stones and gravel / 13·9 sand
Downhill							51·3 silt
33	9·8	4·4	237	5·48	198	4·58	11·6 clay
35	9·8	4·9	258	5·37	219	4·56	
37	9·8	6·0	262	4·45	223	3·80	
39	9·8	8·0	453	5·82	414	5·30	
				Δ av. = 5·28		δ av. = 4·56	

Average for the series: $\Delta = 6\cdot87$; $\delta = 5\cdot10$.

Series E consists of two sets of measurements which were the first made on a stubble. The field had received a dressing of farm yard manure on the surface.

In this series, depth was the varying factor. Five measurements were made uphill and four downhill.

<div align="center">Variation of Draft.</div>

Inspection of Table IV shows a variation from 237 to 529 lbs. in gross draft and 198 to 414 lbs. in nett draft.

Some variation occurs in the value of Δ uphill, but very little in the value of δ. Downhill they both show variation.

The manure on the surface of this field was inclined to collect on the coulter and it is possible that it had a slight influence on the draft at shallow depths. This may explain the diminution in the value of δ with increasing depth over the first few measurements.
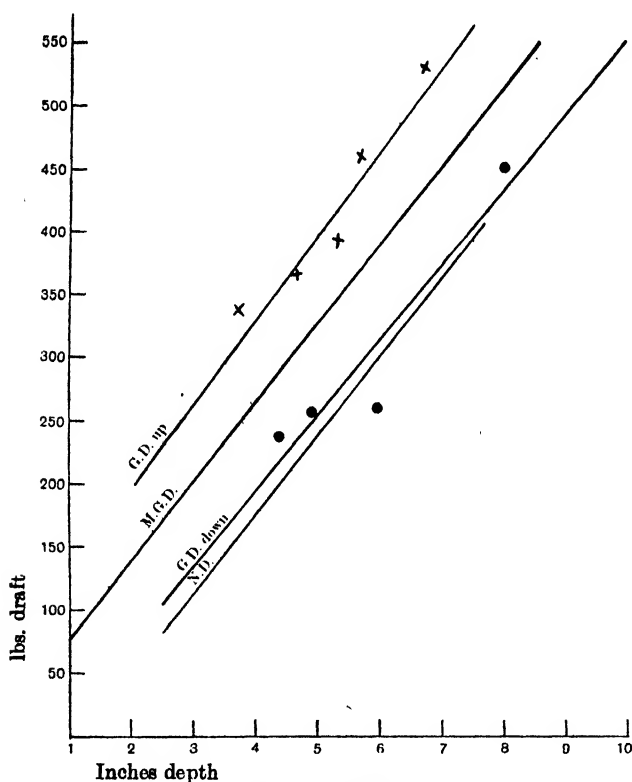
Fig. 5. Series E.

Another point in connection with δ is the difference in value between δ uphill and downhill $(5\cdot58 - 4\cdot56 = 1)$ for which the reason is not apparent.

Fig. 5 shows the draft/depth curve with the points lying close to their respective means.

As in series B, a linear relation seems to exist between depth and draft.

SERIES F.

Table V. Variation of draft with depth.

Chestnut Piece. Ploughing wheat stubble.

Gradient 1 in 7·2. Gradient correction ±54 lbs.

February 21st, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis %
Uphill							
44	9·75	3·9	225	5·99	84	2·23	
46	9·75	5·3	387	7·56	246	4·80	
48	9·75	7·3	417	5·84	276	3·86	21·0 water
			Δ av. = 6·46		δ av. = 3·63		23·6 stones and gravel
							10·2 sand
Downhill							52·5 silt
43	9·0	3·38	195	6·41	162	5·3	15·0 clay
45	9·6	4·01	237	6·15	204	5·29	
47	9·75	5·72	300	5·38	267	4·8	
			Δ av. = 5·98		δ av. = 5·13		

Average for the series: Δ = 6·22; δ = 4·38.

Series F consists of two sets of measurements taken on a wheat stubble in a corner of the field. This was the steepest field on which measurements were taken (gradient 1 in 7·2); depth was made the varying factor. The soil was exceedingly shallow, being only about 7 ins. deep, and one of the charts has had to be left out of account, because the plough share was cutting through the shale in the subsoil. Consequently only three measurements each way are available. Variations from 195 to 417 lbs. are seen in gross draft, and from 84 to 276 lbs. in nett draft. The results show a fairly constant value for Δ in both uphill and downhill measurements. More variation, however, is noticeable in the value of δ than in Δ.

If the average values of δ uphill and downhill be compared, a big difference of 1·5 lbs. is noticeable. This suggests the interference of another factor other than depth and this factor is most likely to be speed.

It was mentioned in the introduction that Professor Davidson (Iowa State University) has already investigated the effect of speed and he has found that an increase of speed from 2 to 3 miles per hour increases the draft from 8 to 12 per cent., and doubling the speed increases the draft 25 per cent.

This being a rather steep field the horses walked much slower uphill, and much faster downhill than their average pace, and this is probably the explanation of the difference in value of δ average uphill and δ average downhill.

Fig. 6 shows the results graphically. The linear relation between depth of furrow and draft is more evident in the downhill measurements than in the uphill measurements.
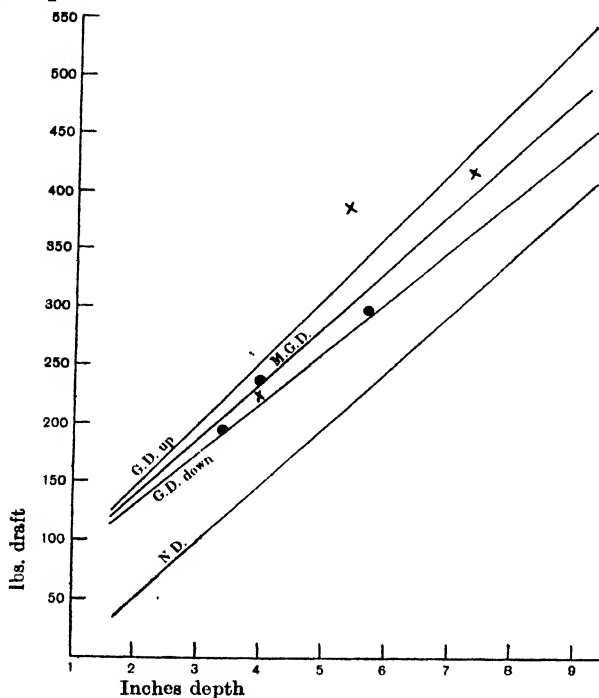
Fig. 6. Series F.

Series G.

Table VI. Variation of draft with depth.

Plum Piece. Ploughing after roots carted off.

Gradient 1 in 8·3. Gradient correction ±40 lbs.

February 22nd, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis %
Uphill							
51	10	3·0	226	7·53	92	3·06	
53	10	4·2	332	7·87	198	4·70	
55	10	6·3	537	8·50	403	6·37	21·3 water
57	10	8·0	580	7·25	446	5·58	32·2 stones and gravel
			Δ av. =7·79		δ av. =4·93		11·5 sand
Downhill							53·4 silt
50	10	3·5	180	5·14	140	4·0	14·1 clay
52	10	4·4	278	6·37	238	5·45	
54	10	6·9	507	7·40	467	6·70	
56	10	9·3	523	5·63	483	5·20	
			Δ av. =6·13		δ av. =5·34		

Average for the series: Δ=6·96; δ=5·13.

Series G (Table VI) consists of two sets of measurements made when ploughing a part of the field where roots had been carted off during the previous autumn.

Depth was again taken as the varying factor and eight measurements were made, four in each direction.
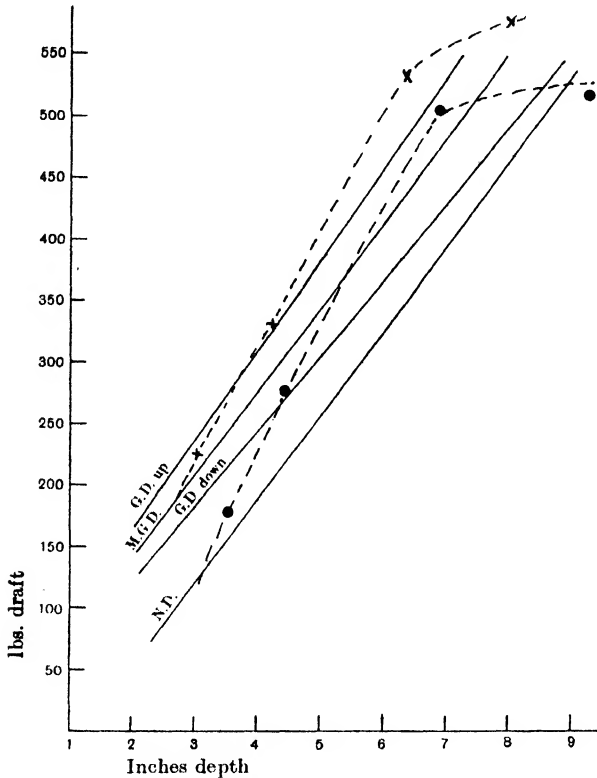
Fig. 7. Series G.

The ground had been consolidated by the passage of horses and carts over it, when the roots were being clamped, and the soil was no doubt very closely packed, particularly on the surface. The moisture content was not noticeably different from that of the previous series.

The gross draft varied from 180 to 580 lbs. and the nett draft from 92 to 483 lbs. It will be noticed that Δ uphill is inclined to be nearer constant in value than Δ downhill. On the other hand, δ shows a considerable and similar variation in both cases, increasing in value up to the third measurement in each set and decreasing in the fourth. This

suggests the operation of an extra factor when a depth of 6 or 7 inches is reached, tending to a relative decrease in the draft. This decrease is believed to be due to looser packing of the soil at that depth resulting from previous deep cultivation, the crust having been hardened by trampling more than the lower parts of the soil. Another point that will be evident is that δ average downhill is slightly higher in value than δ average uphill which may be due to a difference in speed of ploughing.

The results are plotted in Fig. 7, which shows the usual curves and the linear relation between variation of depth and resulting draft, the points being rather scattered about their means.

Inspection of the results of this particular series suggests that the linear relation breaks down (shown by dotted line curves) after a depth of 6 or 7 inches is attained.

<div align="center">SERIES H.</div>

<div align="center">Table VII. Variation of draft with depth.</div>

Five Turnings Field. Ploughing after potatoes.
<div align="right">Gradient 1 in 17. Gradient correction ±23 lbs.</div>
February 22nd, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis %
Uphill							
59	9·5	3·9	317	8·55	207	5·59	
61	9·5	5·6	430	8·08	320	6·01	
63	9·5	7·4	473	6·75	363	5·17	
65	9·5	8·9	537	6·35	427	5·05	23·0 water
			Δ av. =7·43		δ av. =5·45		25·8 stones and gravel
Downhill							16·1 sand
58	9·5	4·6	265	6·03	201	4·56	53·3 silt
60	9·5	5·6	405	7·69	341	6·46	11·3 clay
62	9·5	7·7	500	6·87	436	6·00	
64	9·5	8·7	530	6·43	466	5·66	
			Δ av. =6·75		δ av. =5·67		

<div align="center">Average for the series: Δ =7·09; δ =5·56.</div>

Series H (Table VII) was the last series to be made on a sloping field. The slope was so gradual, only 1 in 17, that the correction needed for this was small.

Variation of draft with depth was again investigated, four measurements being made in each direction.

The previous crop was potatoes lifted in the autumn, during which operation very considerable consolidation of the ground had taken place caused by the passage of men, horses and carts.

Variations from 265 to 537 lbs. in gross draft occurred and from 207 to 466 lbs. in nett draft.

The results do not show any marked tendency in the value of either Δ or δ to be constant uphill or down.

It is very evident, however, that the difference between Δ average uphill and Δ average downhill is small, which would be expected in a field where the gradient is so low.

δ average uphill and down, are in very close agreement, only differing by 0·2 lb., the excess being on the downhill measurements.
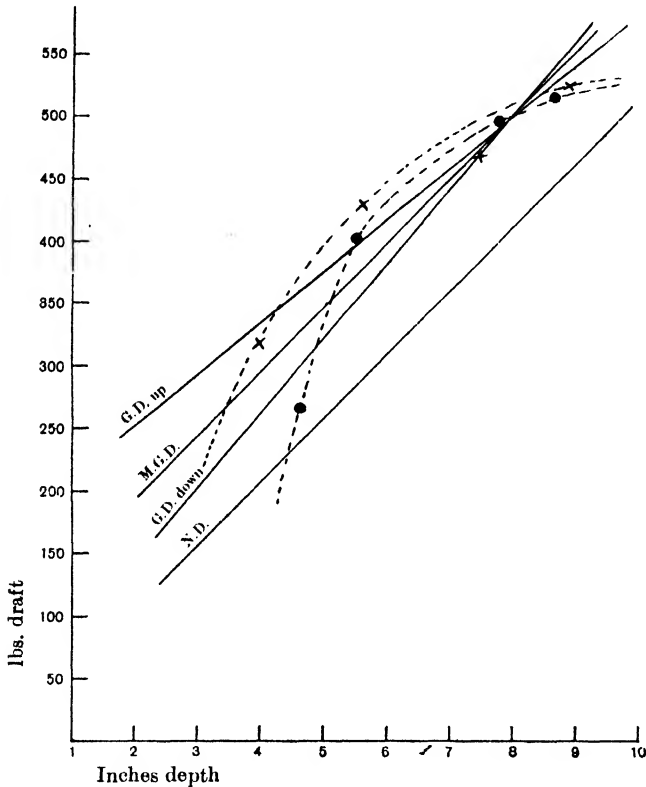
Fig. 8. Series H.

There is nothing in this series to warrant the assumption that δ increases continuously with the depth; in fact after a depth of 5·6 inches is reached, δ appears to decrease. The series has this point in common with series G, except that in the latter the decrease is noticeable at a greater depth.

Fig. 8 shows the results graphically and the rapid convergence of the straight line curves of G.D. which would be expected on a field with a low gradient.

Again there is rather more scatter among the points of the downhill measurements about their mean than among the uphill measurements.

Dotted curves are here shown as in series G, showing clearly how the draft decreases relatively as the plough cuts more deeply.

SERIES I.

Table VIII. Variation of draft with depth.

Twelve Acres. Ploughing second year clover ley. Level.
February 23rd, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis %
66	9	2·8	190	7·58	103	4·03	30·7 water
67	9	3·4	240	7·85	153	5·00	28·2 stones and gravel
68	9	4·2	265	7·06	178	4·7	12·5 sand
69	9	6·5	383	6·55	296	5·06	44·8 silt
70	9	7·6	480	7·05	393	5·74	12·7 clay

Δ av. = 7·22   δ av. = 4·90

Fig. 9. Series I.

Series I (Table VIII) consists of a set of five measurements, taken when ploughing a two-year clover ley. Five charts were made at varying depths.

The part of the field where these measurements were taken stands 1000 feet above sea level and at least 450 feet above the field in which those in series H were taken. Although the mechanical analysis does not suggest it, the soil in series I is undoubtedly lighter than that in series H.

Considerable variation in gross draft occurs from 190 to 480 lbs. and from 103 to 393 lbs. in nett draft.

It will be noted that Δ is particularly constant in value throughout, while δ shows a gradual increase as the depth increases (except in 68). This suggests an increase in the resistance per square inch of furrow slice as depth increases.

The results are plotted in Fig. 9, where only one curve of gross draft is necessary since the measurements were made on the level.

The linear relationship between depth of furrow and draft are particularly well illustrated, and there is no doubt in this case that the curve tends closely to a straight line.

SERIES K.

Table IX. Variation of draft with depth.

Yew Tree Piece. Ploughing up wheat stubble. Level.
February 23rd, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis %
77	9·9	3·9	263	6·82	176	4·52	25·0 water
78	10	5·1	367	7·31	280	5·58	26·6 stones and gravel
79	10	6·8	450	6·62	363	5·34	12·3 sand
80	10	8·3	543	6·51	456	5·48	46·2 silt
							14·1 clay

Δ av. = 6·81 δ av. = 5·23

Series K shows four measurements taken on a level field when ploughing a wheat stubble where the land was known to work rather more heavily than the rest of the farm.

Depth of furrow was again the varying factor. It will be observed that the gross draft varies from 263 to 543 lbs. and the nett draft from 176 to 456 lbs.

The results show Δ to be fairly constant in value, particularly in Charts Nos. 77, 79, and 80. δ, too, shows little fluctuation. The value average δ = 5·23 lbs. is the highest obtained in any of the measurements. This high value is attributable to the rather stiff soil in this field.

The results are plotted in the two lower curves in Fig. 10. The four points lie near their mean curve and the close linear relation between draft and depth is again well illustrated.
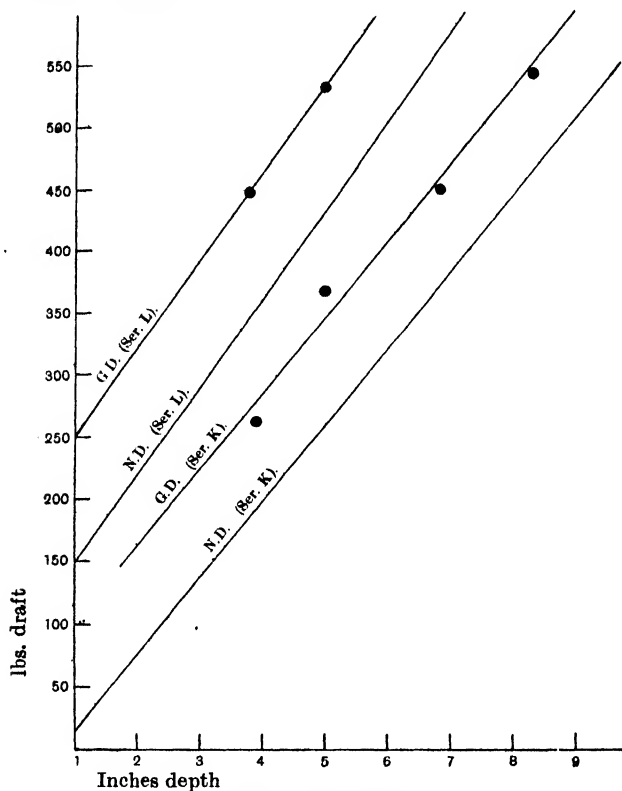
Fig. 10. Series K, L.

SERIES L.

Table X. Measurements with double furrow plough. Variation of depth.

Yew Tree Piece. The furrows adjacent to those in series K. Level.
February 23rd, 1921.

	Width		Depth		Total Draft gross		Total Draft nett		
Chart No.	Front ins.	Back ins.	Front ins.	Back ins.	lbs.	Δ lbs.	lbs.	δ lbs.	Mechanical analysis %
82	9·8	9·8	3·74	3·8	448	6·00	348	4·7	⎧25·0 water
83	9·8	10	4·93	5·1	533	5·34	433	4·37	⎪26·6 stones and gravel ⎪12·3 sand ⎨46·2 silt ⎪14·1 clay
81	—	—	—	—	100	—	—	—	⎩Plough running free on surface

Average for the series: Δ = 5·67; δ = 4·53.

These measurements were carried out with a Roberts' mephisto double furrow plough, with general purpose breasts.

Series L consists of a series of measurements made with a double furrow plough with breasts of the same pattern as the single furrow plough used in the other measurements.

The charts when ploughing were made in furrows adjacent to those in series K, so that the efficiency of the double plough could be tested against that of the single furrow plough.

The double furrow plough presents considerable difficulty when adjusting it to cut two furrows of the same width.

In the two measurements made, closely similar values for δ were found, viz. 4·7 and 4·37 = average 4·53.

When the value average $\Delta = 5\cdot67$ is compared with average $\Delta = 6\cdot81$ in series K, the conclusion is reached that the double furrow plough is a more efficient implement than the single furrow plough.

Chart No. 81 shows the dead weight draft of the double plough and apparatus to be 100 lbs.

The results of series L are plotted in Fig. 10 (the two upper curves). The curve of the nett draft is calculated from the gross draft curve, knowing the draft of the plough and apparatus running free to be 100 lbs.

Second Group. Showing the relation between draft and width of furrow, investigated in Series C, D and J.

SERIES C.

Table XI. Variation of draft with width.

Fifteen Acres. Ploughing one-year clover ley after heavy rain.
Gradient 1 in 9·2. Gradient correction ± 42 lbs.

December 30th, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis
Uphill							
24	6·8	5·4	258	7·00	129	3·50	
18	8·8	5·3	237	5·12	108	2·33	
16	9·9	5·6	245	4·39	116	2·10	
20	10·7	5·6	343	5·78	214	3·60	
22	12·1	5·8	337	4·79	208	2·96	
			Δ av. $=5\cdot41$		δ av. $=2\cdot89$		25·4 water 35·1 stones and gravel
Downhill							15·2 sand 46·9 silt
25	6·8	5·8	186	4·71	141	3·56	11·7 clay
19	9·0	6·1	196	3·56	151	2·74	
17	9·6	5·6	174	3·22	129	2·4	
21	11·0	6·3	250	3·59	205	2·94	
23	12·3	6·6	313	3·90	268	3·30	
			Δ av. $=3\cdot8$		δ av. $=2\cdot98$		

Average for the series: $\Delta = 4\cdot60$; $\delta = 2\cdot93$.

Series C consists of two sets of measurements made on a one-year clover ley after a day and night's heavy rain.

In this field it was decided to measure the effect of variation of the width of the furrow on the draft. It was found much more difficult to keep the depth constant than the width. One reason for this was the uneven nature of the surface. Another reason was that the ground was softer in some places than in others, and as the share of a plough is fixed to cut into the earth, a soft place allows the wheel to cut in more deeply. Also, a plough cutting at a certain mean depth uphill always cuts at a greater mean depth downhill, owing to the position of the horses relative to the plough being lower in the latter case than in the former.
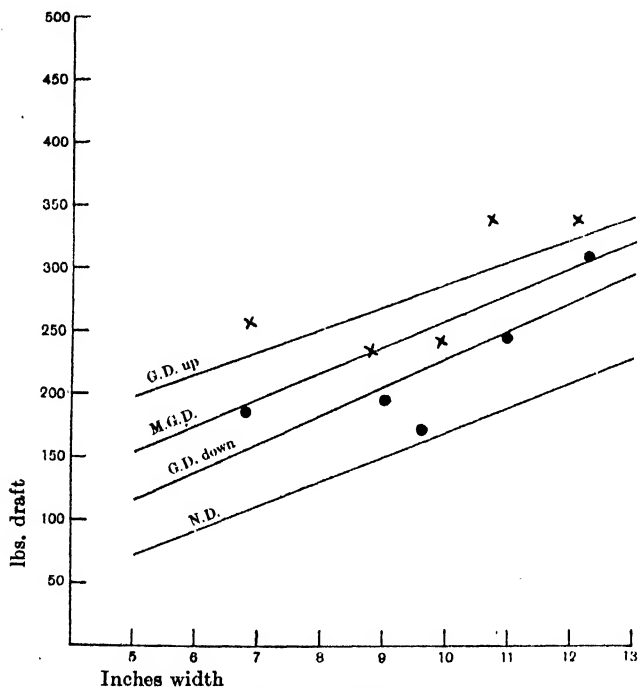
Fig. 11. Series C.

Even with these slightly varying factors, the depths do not show any considerable variation and may be considered constant.

Gross draft shows a variation from 174 to 343 lbs. and nett draft from 108 to 268 lbs.

It will be observed that the average δ uphill is remarkably close in value to average δ downhill.

Fig. 11 shows variations of draft plotted against the variations in width.

Considerable scatter of the points round the mean exists, which

suggests the varying of factors other than width. In a narrow furrow, the base is entirely cut by the share, while in a wide furrow, tearing takes place outside the cutting limits. No doubt this factor must have considerable influence on the draft.·

The highest values of Δ are found in Charts 24 and 25 and these correspond with narrow furrows, which suggests that tearing requires less draft than cutting in this particular soil. This conclusion is justified by measurements described later in series J.

Although the points are somewhat scattered about their means, yet a straight line appears to be the relation between width of furrow and draft. This relationship is more clearly seen in the following series D.

SERIES D.

Table XII. Variation of draft with width.

Fifteen Acres. Ploughing same clover ley as in Series C, but after fine weather.
Gradient 1 in 9·2. Gradient correction ±42 lbs.

February 19th, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis %
Uphill							
26	7·7	5·6	303	7·05	174	4·07	
28	8·2	5·5	302	6·65	173	3·82	19·5 water
30	10·0	5·7	355	6·26	226	3·99	38·4 stones and gravel
			Δ av. =6·65		δ av. =3·96		15·8 sand
Downhill							46·7 silt
27	7·6	6·0	212	4·65	167	3·66	10·8 clay
29	8·6	5·5	262	5·55	217	4·57	
31	10·2	5·8	312	5·26	267	4·49	
			Δ av. =5·15		δ av. =4·24		

Average for the series: $\Delta = 5\cdot90$; $\delta = 4\cdot1$.

Series D consists of two sets of measurements made on the same field as the previous series, and in the same position in the field, but after an interval of nearly two months.

In this series width was again made the varying factor and three measurements were taken in each direction.

The gross draft varies from 212 to 355 lbs., and the nett draft from 167 to 267 lbs.

It will be seen that there is little variation in the value of Δ and δ in the respective columns of the sets to which they belong. Also the average δ uphill is very close in value to average δ downhill, the two only differing by 0·28 lb.

Turning to the curve (Fig. 12) it is evident that the points both uphill and downhill lie very close to their respective mean curves. It is evident also that the relation between width of furrow and draft is linear.

It has already been stated that the greater the area of cross section of the furrow slice, the smaller fraction of the total draft the dead weight draft of the plough becomes; that is, in a sloping field the effect of gravity is felt less at the greater width. Consequently with increase in width or depth, the curves (uphill and downhill) should converge. This argument is supported by the position of the curves in series D.
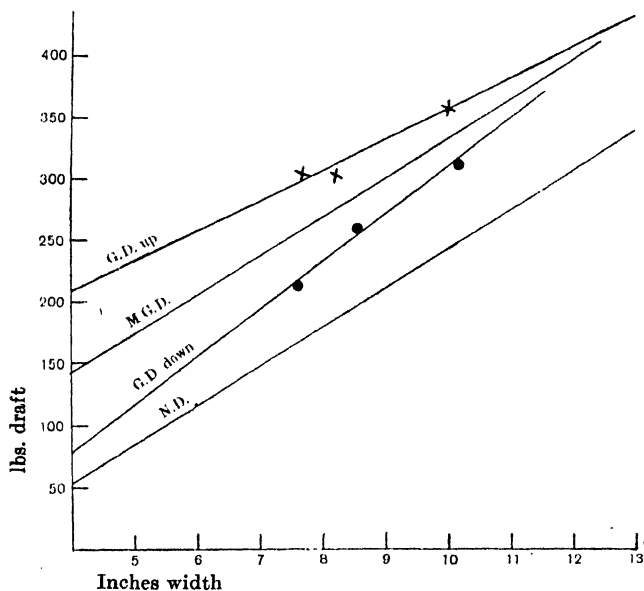
Fig. 12. Series D.

Table XIII. Variation of draft with width.

Twelve Acres. Ploughing second-year clover ley. Level. February 23rd, 1921.

Chart No.	Width ins.	Depth ins.	Draft gross lbs.	Δ lbs.	Draft nett lbs.	δ lbs.	Mechanical analysis %
75	5·4	4·6	236	9·50	149	6·00	30·7 water
74	7·5	4·4	240	7·28	153	4·61	28·2 stones and gravel
71	9·0	4·8	232	5·35	145	3·34	12·5 sand
72	12·3	4·3	320	6·02	233	4·43	44·8 silt
							12·7 clay

Δ av. = 7·04　　δ av. = 4·59

Average for the series: Δ = 7·04; δ = 4·59.

Another important point arising out of a comparison of the results in series C and D is that the drier ground in series D is attended by a much higher draft than the somewhat wetter in series C; or otherwise expressed, a decrease in moisture content of 5·9 per cent. increased the draft 28 per cent., shown by increase in value of average $\Delta = 1\cdot3$ lbs.

SERIES J.

Series J consists of a set of measurements made on the same field as those in series I, and in adjacent furrows, but with width instead of depth of furrow as the varying factor.

The gross draft varies from 232 to 320 lbs. and the nett draft from 145 to 233 lbs.
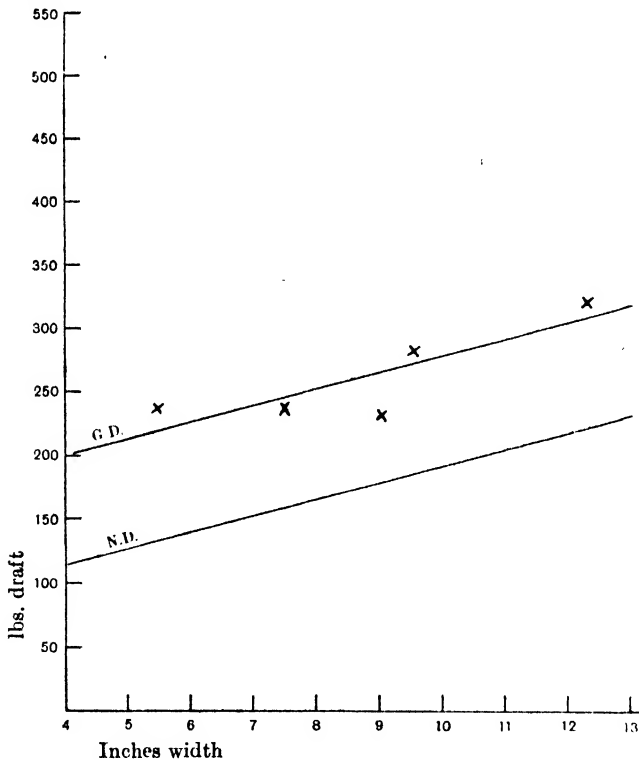
Fig. 13. Series J.

Δ shows considerable fluctuations, its value increasing with decreasing width (except in 71).

The value of δ also shows similar fluctuation, but to a lesser extent. This increasing value of δ (except in 71) with decrease in width of the

furrow suggests that the resistance per square inch of furrow slice is less in wide than in narrow furrows. This fact has been previously noted in series C, and it seems further justification for the suggestion already made, that cutting requires greater draft than tearing in the variety of soil found on this farm.

Fig. 13 shows the results arranged graphically. The points are somewhat scattered about their means, but the curve certainly shows a linear relation between draft and width of furrow.

GENERAL DISCUSSION.

In the description of each series the range of variation of draft has been given.

The gross draft was seen to vary from a minimum 107 lbs. in series B to a maximum 580 lbs. in series G.

At the same time the nett draft varied from 68 to 483 lbs.

When the gross draft for a width of 10 inches and depth of 5 inches (normal ploughing) is obtained from the various mean curves, a variation from 267 (series C) to 350 lbs. is found giving an average of 320 lbs. Similarly for the nett draft a variation from 180 to 263 lbs. is found giving an average of 238 lbs.

The figure 320 lbs. is the average draft of a plough working in a two-horse loam under ordinary farming conditions.

Each horse, therefore, exerts a force equal in weight to about a hundredweight and a half.

THE COMPONENTS OF DRAFT.

It has been mentioned earlier in the paper that the nett draft is utilised for two purposes. One is to cut through the soil, that is, to overcome the resistance of the soil to the coulter and share, and the other is to lift and turn the furrow slice till it rests on its edge.

INFLUENCE OF DEPTH OF FURROW ON DRAFT.

This was investigated in series B, E, F, G, H, I and K, and, within certain limits, a linear relation, in some cases more clearly defined than in others, is seen to exist.

It has just been restated that the nett draft is composed of two components. This being so, it is interesting to discuss shortly, on theoretical grounds, the probable effect of increase in depth on each of these components.

First, what is the effect of doubling the depth on that force required to turn the slice; from the principle of work, since double the weight

is raised through a rather greater height, it follows that a little more than double the work is done in turning a slice twice as thick as the normal. Therefore δ should show a slight increase at double the depth.

Secondly, what is the effect of increase of depth on the force required to cut the slice. Under ordinary conditions the ground is more consolidated at deeper levels than near the surface, so that an increase in δ would most probably result from this cause. Therefore it would be expected that this force, and therefore δ, would increase slightly with increasing depth.

This is found to be so in all cases examined except in series G and H. In these series δ shows a decrease after a certain depth is reached. This, as has already been explained, is probably due to the consolidation of the upper layers rather than the lower layers by the trampling of men and horses and the passage of carts when carting the roots, so that the top layer actually became a crust covering looser soil.
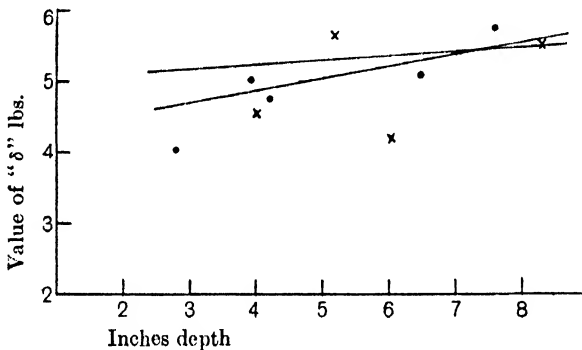
Fig. 14.

On the whole, however, it is clear in these experiments that as the depth increases within the usual limits the value of δ increases slowly. On other soils, the increase of δ with depth might be very considerable, and this seems to be the conclusion reached from observations of draft made at the Tractor Trials at Shrawardine (Salop) in September, 1921, where the soils were much heavier.

The curves in Fig. 14 (series I and K), obtained by plotting the values of δ (ordinates) against the corresponding depths (abscissae) are intended to show the gradual increase of δ as the depth of the furrow increases, showing that the consolidation tends to increase with the depth.

INFLUENCE OF WIDTH OF FURROW ON THE DRAFT.

Consider what would be the effect of increasing the width on the two draft components.

Take first the effect in turning the furrow. It can be shown theoretically that the work done in turning a continually widening furrow increases more rapidly than when turning a deepening furrow; consequently δ, the nett draft per sq. inch, should increase more rapidly with increase in width than with depth. On the other hand, the effort required to cut a wider slice should not be more than twice as great for a furrow twice as wide as a normal furrow, since the consolidation should be the same at constant depth. So theoretical considerations suggest an increase in δ with increase in width.

In the three series C, D and J, δ increases a little with the width in the two former, but decreases considerably in the latter. The explanation for this (in series J) lies most probably in the fact that outside the cutting region of the share the furrow must be torn, and as the width increases so the tearing effect is more felt, and with light soil tearing probably requires less draft than cutting, so that δ at considerable width has a smaller value than δ at a lesser width.

In the other soils (series C and D), while δ certainly increases over part of the series, in both sets of series C it is at its highest value when the furrow is narrowest, but not so in series D.

From the three measurements it is clear that the draft relationship to the width is linear.

INFLUENCE OF VARIATIONS OF BOTH WIDTH AND DEPTH OF FURROW ON DRAFT.

If the curves in series I and J are compared, it will be noticed that the depth/draft curve in series I is much steeper than the width/draft curve in series J.

Since the draft varies with the width and the depth, it will vary with their product, that is, draft $\propto d \times w$, where $d =$ depth and $w =$ width; therefore, the draft varies with the area of cross section of the furrow slice.

In the case of a furrow of constant width 10 ins. (series J), an increase of 1 in. depth increases the area of cross section by 10 sq. ins., but where the furrow is of constant depth 5 ins. (series J) an increase of 1 in. width means only an increase in area of cross section of 5 sq. ins. Therefore, the increase in draft in the first case is double the increase in the

second case, so that the curve in series I will be about twice as steep as that in series J. Therefore, an increase of 1 in. width in ordinary ploughing conditions is only attended by an increase of half the draft that would attend an increase of 1 in. depth, and explains why increasing the depth in the soil makes the work much harder for the horse than the same increase in width.

Influence of Moisture Content of the Ground on Draft (Gross).

It is well known that an optimum moisture content exists at which the draft of a plough in any soil is lowest. This optimum lies between extremes of dryness and wetness, and is considered to be the most suitable state of the soil for good ploughing and satisfactory work. This optimum is probably the point when the pulverising effect of ploughing is most felt.

If series C and D be compared, the effect of loss of moisture in increasing the draft is seen:

Series C.	% moisture* 25·4	$\Delta = 4\cdot6$
Series D.	% moisture 19·5	$\Delta = 5\cdot9$

Decrease 5·9 %. Increase 1·3 % = 28 %.

* Calculated on 100 parts of air dry soil.

So that a decrease in soil moisture of 5·9 per cent. is attended by an increase in draft of 28 per cent.

The optimum moisture content was not found, in fact weather conditions did not permit, there not being sufficient rain in order to determine the draft in very wet soil. But no doubt the figure $\Delta = 4\cdot6$ being low, is not far removed from Δ at optimum moisture content.

Measurements using a Double Plough.

The object was to compare the efficiency of the double furrow plough with that of the single plough.

As already explained when discussing the measurements in series L, a value average $\Delta = 5\cdot67$ lbs. was obtained with the double plough. When this is compared with the value $\Delta = 6\cdot8$ obtained with the single plough in series K, it is clear that the double plough is the more efficient machine. This conclusion is in accord with experience and with the statements made by the agricultural implement manufacturers.

The State of Consolidation of the Ground
after Various Crops.

It has been previously stated that an examination of the soils on the Folley Farm shows a considerable uniformity in texture in every field, and that the measurements (except series B and C) were made at the same time of year, during a spell of dry weather. Therefore, a comparison of the values of the average δ in the various series will give an indication of the consolidation of the ground after the various crops. Only rough indications can, of course, be obtained, since the value of δ can be influenced by so many causes and in another year the order may be modified.
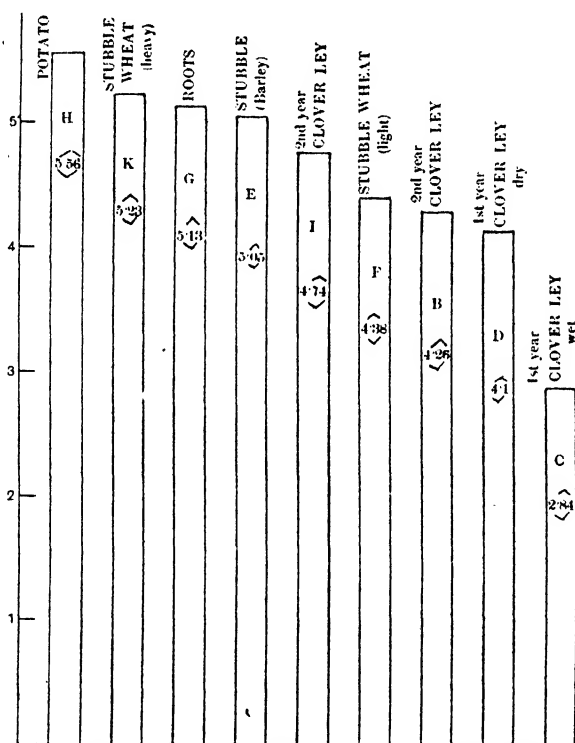
Fig. 15.

The values of δ are shown in Fig. 14, arranged in order of magnitude.

Series H exhibits the greatest consolidation ($\delta = 5\cdot56$) following a potato crop. Series K, taken after wheat on a stiffish field, and series G after a root crop, follow next. Thereafter the various series are shown in order down to C, which has the lowest value of δ ($\delta = 2\cdot84$).

It is noticeable that both root crops are close together at the head of the list, and that the two second-year clover leys stand higher than the one-year clover ley, as would be expected.

The position of the stubbles indicates to some extent the weather conditions prevailing at harvest time.

A comparison of series C and D shows the distinct help of a good downpour in decreasing the draft and making the work lighter for the horses.

SUMMARY OF CONCLUSIONS.

The state of consolidation resulting from the nature and treatment of the previous crop has a most marked effect on the draft, causing variations from 107 to 580 lbs. in gross draft and from 68 to 483 lbs. in nett draft on a typical two-horse soil, the force exerted by each horse being about one hundredweight and a half.

The relation between draft and depth of furrow is linear, the nett draft per square inch tending to increase in value with increasing depth, except when the previous crop was roots, when a decrease at depths greater than 6–8 ins. is noticeable.

The relation between draft and width of furrow is linear, the nett draft per square inch in some cases showing an increase and in others a decrease with increasing width.

Observations suggest that a greater proportion of the nett draft is used in overcoming the resistance of the soil than in turning the furrow.

The quantity of moisture in the soil has a considerable effect on draft. There is probably an optimum content, from which an increase or a decrease would result in increased draft. In the case measured, a decrease in soil moisture of 6 per cent. resulted in an increase in draft of 28 per cent.

The double plough is a more efficient implement than the single plough, fittings and mould-board being similar in design.

Mr G. Udny Yule, C.B.E., M.A., F.R.S., whose assistance and criticism was sought on points of method in calculation.

Mr C. L. Whittles, B.A., who made the mechanical analyses of the soil samples.

Mr H. A. Elliot, Cert.R.San.I., Surveyor to the Clun Rural District Council, who measured the gradients.

Finally, to his brother, Mr H. P. Davies, who always assisted him in making the actual measurements.

(Received 1st December, 1923.)

FIELD EXPERIMENTS.

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(With One Text-figure.)

SINCE 1899, field trials have been carried out at the Experiment Station, Kilmarnock, of the West of Scotland College of Agriculture and in the counties in the administrative area of the college. Their object was (1) to compare the yield of different varieties of oats and turnips, and (2) to determine the best system of manuring different crops grown under varying conditions of soil and climate. Single plot tests were invariably employed and in the interpretation of the results experimental errors were not taken into account as at that time (1902–1909) very incomplete data were available for that purpose. The results were published in college bulletins[1]. As the figures contained therein are often quoted it seemed highly desirable that the extent of the experimental errors in trials of this kind should be available. With this object in view an examination of the results has been undertaken which forms the subject matter of this short paper. Since the Oat Trials provide the largest amount of data, attention was directed principally to this crop.

OAT TESTS AT DIFFERENT CENTRES.

As many as 12 different varieties were sometimes used in a test and from 20 to 35 tests were conducted annually at centres distributed over the counties in the West of Scotland. It has already been pointed out that single plots were invariably employed, a procedure which allowed of the inclusion of the maximum number of varieties with least amount of space and which involved least cost. The seed corn supplied was from the same bulk sample.

The scheme of the experiment was as follows. A member of the college staff selected and laid off the ground and superintended the

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[1] Report on Experiments on the Comparative Merits of Varieties of Oats by R. P. Wright, Second, Third and Ninth Annual Reports, West of Scotland College of Agriculture.

seeding of the plots. College officers inspected the plots at intervals during the course of the experiment. To prevent undue attention of birds during the ripening of the crop, the plots were located in a field of oats. Several standard varieties were included in each test to act as a basis for comparison. The size (1/20th acre) and shape of plot, the weight of seed corn, and the method of harvesting, threshing and weighing of the produce were the same at each centre for each year. The varieties were cut as they ripened and the weight of grain and straw along with other relevant data obtained for each plot.

The statistical method of examination followed was the same as that adopted by Wood and Stratton[1] in their valuable paper on "Errors in Agricultural Experiments" published in a previous issue of this Journal. Frequency curves were first constructed, the number of centres being plotted on the vertical and the yields of grain in lbs. on the horizontal axis. This was done for the three varieties, Potato, Sandy and Banner and the curves are shown in Fig. 1 below.

Potato ——   Sandy – – –   Banner – · –
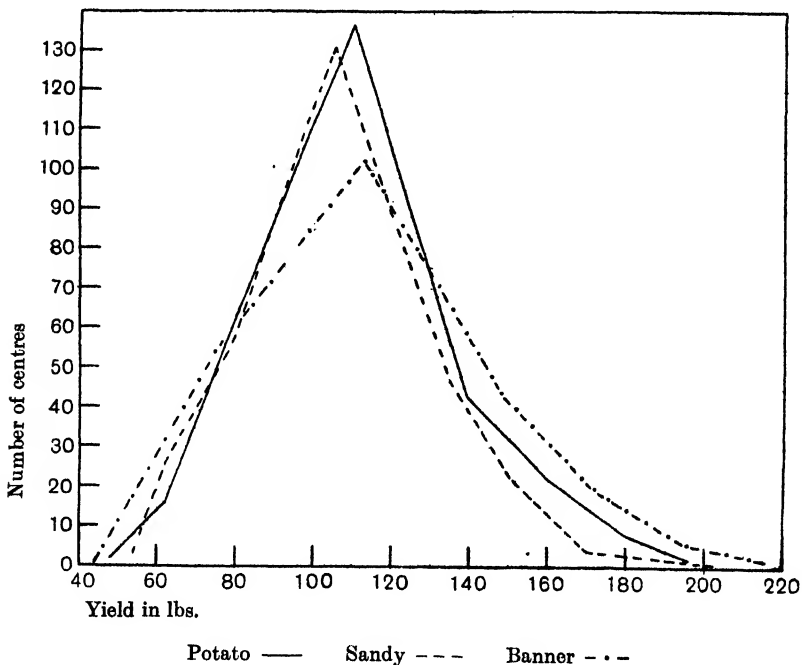
Fig. 1.

[1] Wood, T. B. and Stratton, F. G. M. "The interpretation of experimental results." *Journ. Agric. Sci.* **3**, Part 4.

The three curves appear to conform fairly closely to a normal frequency curve. From this we may assume that we are dealing with a normal variable, and that the figures are suitable for averaging and for the calculation of the probable error. The slight divergence from the normal on the left of each of the three curves, represents the centres with the lowest yields. The cause of this is, no doubt, due to the almost complete elimination of the poorest types of soil in the selection of centres.

The probable error of the average total grain yield for five varieties for each year calculated from the difference of each plot from the average yield of grain was next calculated. The figures obtained are summarised in Table I below. In order to shorten the table as much as possible, the annual figures for one variety only, namely, the Potato oat is included.

Table I.

	Number of centres	Total yield of grain in lbs. per plot			P.e. of av. in lbs.	Percentage p.e. of av. in lbs.
		Average over all centres	Lowest at any one centre	Highest at any one centre		
Potato 1902	35	120	78	183	19·4	16·2
,, 1903	21	104	52	146	18·9	18·2
,, 1904	29	103	60	193	24·0	23·3
,, 1905	32	118	70	179	17·3	14·7
,, 1906	25	110	59	162	19·1	17·4
,, 1907	21	108	63	170	19·2	17·7
,, 1908	30	114	58	192	19·5	17·1
,, 1909	31	136	75	198	19·1	14·0
,, 1902–1909	224	114	52	198	20·6	19·2
Sandy 1902–1909	224	110	58	202	19·5	16·8
Banner 1902–1909	193	117	49	218	24·4	20·8
Mounted Police 1906–1909	107	124	62	227	23·5	19·0
Wide Awake 1906–1909	107	128	57	262	25·6	20·0

Comparing the yields of the old Scotch varieties, namely, Potato and Sandy over a long period and grown under the soil and climatic conditions prevailing in the West of Scotland, it is found that the Potato cat gives an average yield of grain amounting to about 4 per cent. more than Sandy. New varieties such as were in cultivation at that time, *e.g.* Banner, gave a yield amounting to about 6 per cent., Mounted Police 13 per cent. and Wide Awake 16 per cent. above that of Sandy. The last two named new varieties were not introduced into the trials until 1906, and the number of centres yielding figures were 107 compared with over 200 in the other cases. It is probable that if these varieties, namely Wide Awake and Mounted Police had been under test for the full period, their superiority over the older types would approximate to that of Banner.

It is noticeable from the table that the newer varieties show a greater range of variation in the yields of grain compared with the older varieties. The former give higher maximum yields compared with the latter. The elimination of the poorest types of soil in the selection of centres would adversely affect comparisons between the yields of old and of new varieties in so far as the older varieties would do better than the new varieties under poor soil and climatic conditions.

It is a well-established fact that the old Scotch varieties of oats are hardier and can better endure the greater extremes of soil and climate than some of the new varieties. For this reason and from the remarks made above respecting differences in yields, a variation of the experimental error due to variety was to be expected. Examination of the figures in Table I confirms this. The average probable error for the old varieties works out at about 18 per cent. and for the new varieties at about 20 per cent. of the total yield of grain.

Table II below gives the average yield of grain, the probable and the percentage error for Potato oat in each of the counties during the period 1902–1909.

<div align="center">Table II.</div>

County	Number of centres	Average yield of total grain per plot	Average grain yields in lbs.	
			Probable error	Percentage error
		lbs.		
Ayrshire	46	120	19·35	16·13
Argyllshire	27	121	19·61	16·21
Dumfriesshire	30	108	19·40	17·96
Dumbartonshire	26	103	19·05	18·50
Lanarkshire	36	107	20·67	19·32
Stirlingshire	30	118	20·59	17·45

According to the above table the error for each county is similar to that for the combined counties. This means that the climatic and soil conditions met with in any of the counties in question would appear to be as diverse as that obtained in the whole of the West of Scotland, in so far as they affect the Potato oat.

From the foregoing figures the probable error on single plot trials for the old Scotch varieties of oat is about 18 per cent. and for the new varieties about 20 per cent. of the yield of grain. Adopting the recognised method of calculation, this means that when experimenting in any county in the West of Scotland with two varieties of oats which are only likely to show a difference in their grain yields amounting to about 5 per cent., it is necessary to have 214 centres with no duplication of plots at any centre in order to endeavour to obtain a conclusive result

from a single year's trials, and 53 centres where the varieties under test differ in yielding power by 10 per cent.

Taking Potato oat as the standard type, it is seldom found that any other variety will surpass it in yielding power by more than 10 per cent. under the varying conditions of soil and climate that obtain in the West of Scotland. Banner surpasses it in yield of grain by from 2 to 3 per cent.

Converting the yield of grain per plot into yield per acre it is interesting to note that the following figures are obtained for the varieties in question.

Sandy ...	...	52 bushels per acre
Potato	...	54 ,, ,,
Banner	...	56 ,, ,,
Mounted Police	59	,, ,,
Wide Awake ...	61	,, ,,

OAT TESTS AT THE SAME CENTRE.

Using duplicate plots, Wood and Stratton found the probable error to be 5 per cent. of the yield of grain, whilst Hall and Mercer[1] showed that with 1/40th acre plots repeated five times the error was reduced to 2 per cent. It is possible, however, that the probable error is not the same for each crop. Allowance must be made for individual characteristics of crops in so far as they are affected by methods of sowing, cultivation, harvesting, immunity or otherwise to fungoid and insect pests, shedding of grain, etc. The cumulative effects of these will operate in varying degrees according as they apply to each crop. The following figures provide some data on this point. The method of calculation employed to arrive at the figures is as follows. The mean yield of duplicate plots is obtained and the difference of each plot from the mean is expressed as a percentage of the mean and the error then calculated in the usual way.

1/40th acre plots.

	Number of duplicate plots	Probable error of duplicate plots %
Oats 1910–11 grain	55	6·0
,, ,, straw	55	4·2
Hay ,,	28	1·2
Potatoes	19	3·4

Although the experimental data from which the above calculations are made are too few to arrive at a real comparison of the errors for different crops, nevertheless they are sufficient to indicate that differences in the probable errors do exist.

[1] Mercer, W. B. and Hall, A. D. "The experimental error of Field Trials." *Journ. Agric. Sci.* **4**, p. 2.

SUMMARY.

Single plot trials owing to the magnitude of the experimental errors, are practically useless as a test for comparing the yields of grain of one variety of oat with that of another. At best the results are only applicable to the particular experiment in question. For the old Scotch varieties of oat the probable error on trials of this kind amounts to about 18 per cent. of the yield of grain and to about 20 per cent. for the new varieties. Adopting single plot trials an 18 per cent. error means that when determining the superiority in the yield of grain of one variety over that of another and when the difference is not likely to be more than about 5 per cent. it would be necessary to have 214 centres with no duplication of plots at any centre in order to endeavour to obtain a conclusive result from a single year's trials. When the difference is likely to be about 10 per cent. it would be necessary to have 53 centres.

Data are supplied showing that the experimental error for different crops also for the grain and straw of the same crop is not the same. It is greatest for the grain and lowest for the hay crop.

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A METHOD FOR THE ESTIMATION OF URIC ACID IN POULTRY EXCRETA.

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INTRODUCTION.

IN connection with nutritional investigations on poultry being carried out in the above Institute by the writer's colleague, Mr E. T. Halnan, it became necessary to devise a satisfactory method for the estimation of uric acid in poultry excreta. Although numerous exact methods have been elaborated for the determination of the amount of uric acid in mammalian urine, the corresponding problem in connection with the excreta of birds has received relatively little attention. This circumstance is no doubt due in large measure to the peculiar difficulty attaching to the quantitative investigation of the nitrogenous constituents of poultry excreta, arising from the fact that the urine and faeces are not excreted separately. The urine is not temporarily stored in a bladder, as with mammals, but proceeds directly from the kidneys through the ureters to the cloaca and there undergoes admixture with the faeces from the intestines.

The most abundant and characteristic nitrogenous constituent of avian urine is not urea but uric acid. The latter is present as the insoluble ammonium biurate, probably mixed with some free uric acid [1]. Uric acid behaves in aqueous solution as a monobasic acid and consequently only the acid salts (biurates) exist in aqueous solution. In the presence of strong bases, both hydrogen atoms may be replaced with the formation of neutral urates. The latter, however, are only stable in solutions containing an excess of the base. They are readily decomposed by water to give the biurate.

In addition to these two series of urates, Scherer[2] claimed to have demonstrated the existence of a third type of urate in the urate sediment of urine, namely, the so-called quadriurates, consisting of a molecule of biurate united with one of uric acid. The quadriurates were stated to be

more insoluble than the corresponding biurates, and Roberts (3) formed the conclusion that the uric acid in the renal excretion of birds was present in the form of ammonium quadriurate.

The later researches of Tunnicliffe and Rosenheim (4), however, have made it clear that the quadriurates have no definite existence and are to be regarded merely as mixtures of biurate and uric acid.

OLD METHODS OF ESTIMATING URIC ACID IN POULTRY EXCRETA.

The earliest method in use for this purpose was that of von Knieriem (5), which involved the extraction of the excrement with boiling caustic soda or potash. The filtered extract plus hot water washings were acidified and evaporated to a small bulk. The uric acid which separated on standing was filtered off and its amount determined gravimetrically or by means of the Kjeldahl method.

This crude method was open to the objection that when uric acid is precipitated from such alkaline extracts, it is deeply pigmented and is contaminated with foreign organic material (*e.g.* protein) which interferes with its estimation either by the gravimetric or the Kjeldahl method.

A marked advance was made as a result of the investigations of Tunnicliffe and Rosenheim (6), who demonstrated that pure uric acid could be titrated quantitatively against $N/20$ piperidine solution. Piperidine is a fairly ready solvent for uric acid, the soluble piperidine urate being formed in the process.

The method of Brown (7), subsequently modified and improved by Bartlett (8), was based on the preceding discovery. The procedure consisted in the first place of removing the pigment as completely as possible from a weighed amount of excrement (10 gm.) by successive washings with alcohol and ether. The dried residue was acidified with 0·5 per cent. hydrochloric acid and allowed to remain overnight in a refrigerator. After filtering and washing with water, the material was extracted with piperidine on the steam bath. The extract was filtered through coarse linen into a 500 c.c. measuring flask and after well washing the residue on the linen with hot water, filtrate and washings were made up to the mark and the suspended material was allowed to settle overnight. 50 c.c. of the clear extract was next made acid with hydrochloric acid and evaporated to 25 c.c. on the water bath. The uric acid which separated on cooling and standing was filtered off, washed with cold water and finally with alcohol and ether to remove traces of fat. It was subsequently transferred to a beaker, boiled with 35 c.c. water and titrated with $N/10$ piperidine

in the presence of phenolphthalein, the end point reaction being completed as near as possible to the boiling point.

Although the mean of several determinations carried out on pure uric acid by Bartlett showed that 98 per cent. of the acid could be recovered in this process, yet it is clear that the application of a method which relies mainly on the use of a solvent like piperidine, with its pungent peppery smell, cannot be entirely satisfactory, especially in view of the lengthy extraction on the steam bath (30–45 minutes) which was necessary to effect complete solution of the uric acid. Furthermore, the piperidine extracts were always difficult to filter, the process frequently being quite impossible. For this reason, Bartlett had recourse to filtration through linen, the filtrate being allowed to stand for many hours in order that a clear extract could be obtained by settlement. It was also essential that the final titration with $N/10$ piperidine should be carried out in nearly boiling solution and a satisfactory end point could not be obtained unless the pigment of the excrement had been thoroughly removed by the initial alcohol-ether treatment, frequently a difficult matter to accomplish with poultry excreta. Moreover, if the uric acid crystals had not been thoroughly freed from hydrochloric acid during filtration and subsequent washing, then a very appreciable error resulted in the value of the titration figure.

A point which was rightly urged in favour of the method was that the presence of protein did not necessarily interfere with the accuracy of the final titration.

For the purpose of estimating the amount of uric acid in avian urine, Kossa[9] utilised the property of uric acid of dissolving in warm concentrated sulphuric acid. Whilst, however, this method may be quite satisfactory when dealing with the separately collected renal excretion, the experience of the writer showed that the extraction of the mixed excreta yields a fluid of treacly consistency which cannot be filtered by any method.

GENERAL OUTLINE OF THE NEW METHOD.

The ready solubility of lithium urate led the writer naturally to turn his attention to the use of lithia as a solvent for extracting the uric acid from poultry excreta. A method was elaborated which in its finally adopted form consisted of the following six stages:

(1) Removal of pigment from weighed amount (8–10 gm.) of excrement by successive treatment with cold alcohol and ether.

(2) Decomposition of ammonium urate in excrement by means of hydrochloric acid.

(3) Extraction of uric acid by means of lithia solution.

(4) Precipitation of uric acid from lithia extract by means of ammonium chloride.

(5) Decomposition of ammonium urate obtained under (4) by means of hydrochloric acid.

(6) Determination of amount of uric acid by direct weighing or titration with $0.05\ N . \mathrm{KMnO_4}$.

It will be noted that from stage (4) the method is substantially an extension of the well-known Hopkins method [10] of determining the amount of uric acid in mammalian urine. If a sample of the latter be saturated with ammonium chloride, the whole of the uric acid separates as ammonium urate on standing. The precipitation is only complete when the urine is saturated, or nearly saturated, with ammonium chloride, but under these conditions, the reaction is so perfect, that it is impossible by any tests to demonstrate the presence of uric acid in the filtrate. Hopkins has shown that not only uric acid, but also pigments and xanthin are precipitated in this manner. Creatinine and hypoxanthin, on the other hand, remain in solution. During the subsequent decomposition of the ammonium urate with hydrochloric acid, most of the pigments and all the xanthin are removed, so that the presence of xanthin does not affect the accuracy of the method.

Protein appears to have little or no influence on the accuracy of the method. It is only incompletely precipitated by the ammonium chloride, and the small amount of precipitated protein is removed during the treatment of the precipitate with hydrochloric acid and in the subsequent washing.

Experiments carried out by Hopkins on pure uric acid showed that by this means the estimation of uric acid in mammalian urine could be carried out with an experimental error of not more than 1 per cent.

PRECIPITATION OF URIC ACID FROM LITHIA SOLUTION BY MEANS OF AMMONIUM CHLORIDE.

The addition of ammonium chloride to a solution of any alkaline biurate produces a precipitate of ammonium biurate. Thus, if ammonium chloride be added to a solution of uric acid in lithia, an immediate precipitate of ammonium urate is obtained. Under normal circumstances, however, it would be anticipated that this reaction, in the presence of free lithia, would not proceed perfectly to completion. For the success

of the method, it was essential to regulate the conditions of the reaction in order to ensure that the separation of the ammonium urate should take place quantitatively. With the object of securing this result, the amount of lithia used to dissolve the uric acid was kept as low as possible, whilst the actual precipitation was effected in the presence of an overwhelming excess of ammonium chloride. The following description summarises the conditions for quantitative separation of the uric acid.

A weighed amount of uric acid (0·1 –0·2 gm.) was dissolved in a mixture of 45 c.c. distilled water and 5 c.c. of a 5 per cent. solution of lithia. Into the clear solution was stirred 15 gm. of recrystallised ammonium chloride and the mixture was allowed to stand overnight. The precipitated ammonium urate readily settled to the bottom of the beaker and the supernatant liquor was water clear. The latter possessed a strong ammoniacal smell, showing that the excess of lithia originally present had reacted with ammonium chloride to produce ammonia and lithium chloride. Little or no free lithia could therefore be present in the reaction mixture. The presence of free ammonia constituted a distinct advantage, since it is customary to add this reagent when precipitating uric acid from mammalian urine by means of ammonium chloride in order to accelerate the separation of the ammonium urate. Crouson and Vilaret(11) state that the presence of free ammonia is necessary to ensure a perfectly quantitative separation of ammonium urate by the Hopkins method.

The precipitate of ammonium urate was filtered on to a small hardened filter paper and washed with a little saturated ammonium chloride solution. It was then washed back into the beaker by means of a fine jet of hot water, using about 50 c.c. of water. The suspension was carefully brought to the boil and at this stage 5 c.c. of concentrated hydrochloric acid were run in from a pipette. The precipitate went completely into solution and uric acid crystallised out on cooling. The bulk of the liquid was reduced to 20 c.c. on the sand bath and, after standing overnight, the uric acid was filtered on to a weighed Gooch filter. It was then washed with a little distilled water until free from chloride, dried in a steam oven and weighed.

The following results are typical of several determinations carried out in the above manner:

	(1)	(2)
Weight of uric acid taken	0·1120 gm.	0·1552 gm.
„ „ recovered	0·1112	0·1534

It is therefore clear that in the presence of only a slight excess of lithia and a large excess of ammonium chloride, uric acid can be pre-

cipitated quantitatively as ammonium urate from its solution in lithia. If, however, the amount of lithia used is excessive, the precipitation is not perfectly quantitative. For example, 0·1563 gm. of uric acid was dissolved in a mixture of 20 c.c. of 5 per cent. lithia and 30 c.c. distilled water. The amount of uric acid recovered by saturating the solution with ammonium chloride and decomposing the ammonium urate so obtained with hydrochloric acid was only 0·1475 gm.

In order further to test the quantitative character of the reaction, the following experiment was carried out. 1 gm. of uric acid was dissolved in a mixture of 90 c.c. water and 10 c.c. of 5 per cent. lithia, gentle heat being employed to effect complete solution. The concentration of uric acid in this solution was therefore three or four times as great as it is ever likely to be in lithia extracts of poultry excreta as prepared according to the method to be described later. The solution was saturated with ammonium chloride, allowed to stand overnight and filtered.

A portion of the clear filtrate was allowed to stand over a period of three weeks. During this time no further separation occurred.

Another portion was diluted with water and acidified with dilute hydrochloric acid. No precipitate was produced by this treatment.

The remainder of the filtrate was evaporated to dryness on a sand bath and the residue was submitted to the murexide test. A negative result was obtained.

It follows therefore that even when uric acid is present in lithia solution in relatively large amount, it can wholly be removed as ammonium urate by saturation with ammonium chloride.

EXTRACTION OF URIC ACID FROM POULTRY EXCRETA.

Since the uric acid of poultry excreta is mainly in the form of ammonium urate, it followed that it could be extracted as lithium urate in one of two ways:

(1) Direct extraction by boiling the excrement with lithia.

(2) Initial decomposition of the ammonium urate by means of hydrochloric acid and subsequent solution of the uric acid so formed by means of lithia.

The use of the first process would effect an appreciable simplification of the method, since it would do away with the necessity of treatment of the excrement with hydrochloric acid. In order to test its feasibility, the following experiment was carried out.

0·1885 gm. uric acid was dissolved in lithia and precipitated as ammonium urate under the conditions already outlined. The precipitate

was washed back into the beaker after filtration. To the boiling suspension of the material in 75 c.c. water was added 10 c.c. of 5 per cent. lithia and the bulk was reduced to 25 c.c. on a sand bath. After this prolonged treatment, the ammonium urate was still not completely dissolved and a further addition of 5 c.c. of the lithia solution was necessary to bring this about. The amount of uric acid in the lithia solution was now determined by the method already described. It was found to be 0·1730 gm., *i.e.* 91·8 per cent. of the uric acid originally taken was recovered.

The low results obtained in this and similar experiments led to the abandonment of the method of direct extraction of uric acid by boiling the excrement with lithia solution. The reaction involved does not proceed sufficiently readily and requires too large a concentration of lithia to carry it to completion.

The alternative method of initial acidification and subsequent extraction with lithia proved satisfactory in every respect. The reactions involved not only proceed readily but are also quantitative in character, and the amount of lithia required to effect solution can be kept to a satisfactory low quantity. A detailed account of this part of the method will be found in the descriptions which follow.

ESTIMATION OF URIC ACID IN A MIXTURE OF SHEEP FAECES AND AMMONIUM URATE.

In the preceding paragraphs it has been demonstrated that when ammonium urate has been brought into solution as lithium urate by the twofold process involving acidification and subsequent extraction with lithia, it is further possible to recover the ammonium urate quantitatively by saturating the lithia solution with ammonium chloride. In order to ascertain whether these processes could form the basis of a method for estimating the uric acid in poultry excreta, it was necessary in the first place to test the method on uric acid-free faeces to which had been added a known weight of ammonium urate. In this way the completeness of the recovery of uric acid could be submitted to trial under conditions approximating to those obtaining in the analysis of poultry excrement. For this purpose, mixtures of sheep faeces and ammonium urate of known purity were employed.

To 0·5 gm. of dried powdered sheep faeces was added a weighed amount of ammonium urate (0·1–0·3 gm.) and the two intimately mixed. 25 c.c. distilled water were added, the mixture stirred and brought to a gentle boil, at which point 5 c.c. of concentrated hydrochloric acid was

run in. The boiling and stirring were continued for about a minute, and the beaker was then set aside overnight in a cool place. The contents of the beaker were filtered, and after washing with a little cold water, the residue was washed back into the beaker with 50 c.c. of hot water. To the boiling mixture was now added 5 c.c. of 5 per cent. lithia. The boiling was continued for about a minute and the liquid was well stirred during the process. The lithia extract was then filtered off and the residue was washed with small portions of boiling lithia (using in all 50 c.c., made up by diluting 5 c.c. of 5 per cent. lithia to this volume) and finally with boiling water, until the washings were no longer alkaline.

The extract was evaporated to 100 c.c. and, after cooling somewhat, was saturated with ammonium chloride (30 gm.). The ammonium urate was filtered off after standing overnight and was decomposed with hydrochloric acid in the usual manner. The uric acid was finally collected on a prepared Gooch filter. Before the final weighing, however, the material was washed with a little ether to remove traces of fat arising from the sheep faeces.

The following typical results show that uric acid can be estimated with accuracy by this method in mixtures of sheep faeces and ammonium urate.

	(1)	(2)
Amount of uric acid in ammonium urate taken	0·0994 gm.	0·2332 gm.
„ „ recovered	0·0988	0·2320

Application of Method to Poultry Excreta.

When the method described above was applied to the estimation of uric acid in poultry excreta, it at once became apparent that the pigment of the excrement would prove a disturbing factor. It became necessary to submit the excrement to a preliminary treatment with the object of removing the bulk of the colouring matter. This object was attained by successively extracting the excrement with alcohol and ether, although it was found that the entire removal of the pigment was not readily effected.

The weighed out excrement (8–10 gm.) was stirred with about 30 c.c. of rectified spirit and allowed to stand for 15 minutes, after which time the solvent was poured off through a small hardened filter paper. This process was repeated twice and the residue was similarly extracted with small portions of ether, until the solvent when poured off was only faintly coloured. The extracted residue was freed entirely from ether and the uric acid content was determined under the conditions described in detail in the next section.

The figures given below and in a succeeding section illustrate the good agreement obtained in duplicate determinations by this method. The agreement is eminently satisfactory when considered in conjunction with the difficulty of obtaining a perfectly homogeneous sample of excrement.

The colour of the uric acid as finally weighed was subject to variation from white to light brown in the several trials carried out on different samples of poultry excreta. In view of this circumstance, it was decided to check the purity of the discoloured samples in the following way. The material was washed back from the Gooch into a small beaker with 80–100 c.c. hot water. 10 c.c. of 5 per cent. lithia were now added and the uric acid dissolved by means of gentle heat. The lithia extract was washed by means of distilled water into a 250 c.c. measuring flask, made up to the mark and well mixed. After settlement of the asbestos, 100 c.c. of the clear solution were pipetted off and titrated with $0.05 \, N . KMnO_4$ in the manner to be described in the next section. In all cases where the uric acid was discoloured, the result obtained by titration was slightly lower than that obtained by direct weighing. This fact is illustrated by the following typical figures.

Percentage uric acid in moist excrement	Duplicates		Duplicates		Duplicates	
	(1)	(2)	(3)	(4)	(5)	(6)
(a) By direct weighing	2·51 %	2·45 %	2·35 %	2·38 %	2·05 %	2·09 %
(b) By KMnO₄ titration	2·44 %	2·38 %	2·33 %	2·35 %	2·00 %	2·04 %

If, therefore, the uric acid when filtered on to the Gooch filter in the final stage is at all discoloured, it is safer to rely on the estimation by titration with standard permanganate solution than by direct weighing. In the latter case, the result may be slightly on the high side. An obvious advantage attaching to the titration method is the great saving of time and labour involved.

DETAILED DESCRIPTION OF METHOD.

The excrement is thoroughly mixed by means of pestle and mortar and a sample of 8–10 gm. of the moist homogeneous mass is weighed into a beaker of 180–200 c.c. capacity, preferably provided with a lip. The sample is then freed from colouring matter in the manner described in the preceding section by successive treatment with alcohol and ether. After complete removal of ether, the small amount of material on the filter paper is washed back into the beaker containing the bulk of the extracted excrement by means of a jet of hot water, about 35 c.c. of water being used for the purpose (see Note 1).

The contents of the beaker are now thoroughly mixed by stirring and brought gently to the boil. At this point 5 c.c. of concentrated hydrochloric acid are run in from a pipette, the heating and stirring being continued for about a minute. The volume of the liquid is then reduced to 20 c.c. on the sand bath and the beaker is set aside overnight in a cool place (see Note 2).

The acid liquid is next poured through a small folded filter paper and the residue washed with a little cold water to remove excess of hydrochloric acid (see Note 3). The material is once more washed back into the beaker with about 40 c.c. hot water (see Note 4) and after bringing to a gentle boil, 5 c.c. of 5 per cent. lithia are added and the boiling and stirring continued for about a minute. Immediately after settlement of undissolved material, the lithia solution is filtered off through a fluted filter paper (see Note 5) and the residue is well boiled out with small portions of dilute lithia, using for this purpose a mixture of 45 c.c. water and 5 c.c. of 5 per cent. lithia. This very dilute lithia is also used to extract the small filter paper on to which the acidified excrement had been filtered in the previous operation. The washing is finally completed with boiling distilled water until the liquid coming through the filter no longer reacts alkaline.

The filtrate and washings, which measure about 150 c.c., are now evaporated to 100 c.c. on the sand bath. This evaporation, however, is not actually essential and is merely designed to secure economy in the use of ammonium chloride in the succeeding stage. During the process of concentration, a little lithium carbonate may separate out, but this does not interfere with the estimation of the uric acid, since it readily dissolves again in the hydrochloric acid added in the last stage of the method.

The concentrated liquid (100 c.c.) is allowed to cool somewhat and 30 gm. of ammonium chloride are stirred in (see Note 6). After allowing to stand overnight, the ammonium urate is filtered on to a small hardened filter paper and washed with a little saturated ammonium chloride solution (see Note 7). Whilst still wet, it is washed back into the beaker with about 50 c.c. of hot water and after bringing to the boil, 5 c.c. of concentrated hydrochloric acid are run in. This treatment should result in the ammonium urate going immediately and completely into solution, and uric acid readily crystallises out on cooling.

The volume of the liquid is now reduced to 20 c.c. on the sand bath and after standing overnight, the crystals of uric acid are filtered on to a weighed Gooch filter and washed with a little cold water till free from

chloride (see Note 8). After drying in the steam oven for two hours, the material is washed with a little ether to remove any traces of fat which might be present (see Note 9). It is then dried to constant weight.

If, however, the uric acid is at all discoloured, it is better, for reasons already enumerated, to complete the estimation not by direct weighing but by titration with permanganate (see Note 10). The contents of the Gooch after filtering and washing are transferred back to the beaker and dissolved in dilute lithia, the solution being made up to 250 c.c. as described in a preceding section. 100 c.c. of the clear alkaline extract are pipetted into a conical flask and to this is added 20 c.c. of concentrated sulphuric acid. The *hot* liquid is titrated forthwith with $0.05 N . KMnO_4$ (see Note 11). In calculating the amount of uric acid, the following factor may be used:

$$1 \text{ c.c. } 0.05 \text{ KMnO}_4 = 0.00375 \text{ gm. uric acid.}$$

It is, however, more satisfactory to standardise the permanganate previously against pure uric acid (see Note 12).

NOTES ON THE METHOD.

Note 1. Uric acid is insoluble in both alcohol and ether. In order to be assured of the insolubility of ammonium urate in these solvents, a known weight was submitted to extraction with alcohol and ether. The residue was decomposed in the usual manner with hydrochloric acid and the uric acid collected on a Gooch and weighed.

Weight of uric acid in ammonium urate $= 0.1700$ gm.
,,   ,,   recovered $= 0.1694$

Note 2. Under these conditions the uric acid is entirely insoluble. Qualitative tests carried out on the acidic filtrate showed that the latter contained no dissolved uric acid.

Note 3. An ordinary Townson and Mercer filter paper (diam. 3 ins.) was used in this process.

Note 4. If the material after washing back into the beaker is still very acidic, it is advantageous to bring almost to neutrality with dilute soda before extracting with lithia. In this way, the use of an excessive amount of lithia is avoided. It is also important to wash back the residue into the beaker before it begins to dry on the filter.

Note 5. An ordinary Townson and Mercer filter paper (diam. 6 ins.) was found to be satisfactory for this purpose.

Note 6. If the uric acid is to be determined gravimetrically, then the ammonium chloride used to saturate the lithia extract must contain

no insoluble material. It is advisable, therefore, to use ammonium chloride which has been dissolved in hot water, filtered and allowed to crystallise out again.

Note 7. The ammonium urate precipitate separates in a bulky and flocculent condition. It settles very readily and is easily filtered off and washed.

Note 8. In arriving at the weight of uric acid, it is customary to add on 0·001 gm. for every 15 c.c. of acid mother liquor and not to allow anything for the wash water. In view of the fact that the opinion formerly widely held that uric acid was more soluble in aqueous solutions of strong acids than in water is erroneous, the writer does not consider it necessary to make any allowance for the solubility of uric acid in the mother liquor, provided the volume of the latter does not exceed 20–25 c.c.

Note 9. In the initial experiments, the writer washed the moist uric acid on the Gooch after removal of chlorides first with alcohol and then with ether. Frequently, however, the effect of the alcohol was to cause the precipitate to become somewhat sticky, and for this reason the process was modified as described. At all events, the amount of fat present at this stage must be exceedingly small, in view of the initial treatment of the excrement with ether and the subsequent action of acid and alkali.

Note 10. In the larger number of trials made by the writer, the uric acid when collected on the Gooch was slightly discoloured, and recourse was therefore had to the permanganate titration method for the estimation of the amount of uric acid.

Note 11. If $0·05 N . KMnO_4$ be run into a solution of uric acid in warm dilute sulphuric acid, then the uric acid is readily oxidised to allantoin and the colour of the permanganate disappears. At first every drop of permanganate is decolorised at once and the pink colour does not diffuse even momentarily throughout the liquid. The end point is reached as soon as a drop of permanganate produces a pink flush throughout the liquid. Some practice is required in order to enable the experimenter to decide when this point has been reached, since the pink colour only remains for a short time, and after its disappearance, the further addition of another drop of permanganate restores the pink colour. This disappears in its turn on standing and the process can be repeated for some time. The first pink flush must therefore be carefully looked for.

Before estimating the amount of uric acid by this method, it is of obvious importance to wash the crystals free from chlorides.

The titration with permanganate can further be carried out directly on the ammonium urate precipitate. This proceeding, however, does not result in any appreciable economy of time or labour, since the ammonium urate must first be washed free from chloride by means of ammonium sulphate solution (9 volumes of saturated solution to 1 volume of water). Not only is this a difficult and tedious process, but it is also of importance that the ammonium sulphate be free from ferrous salts, an impurity which is invariably present. The removal of iron is not a simple matter and depends on treatment with ammonium sulphide.

Note 12. The writer used the following method to standardise the permanganate solution against uric acid.

Approximately 1 gm. of ammonium urate was weighed out into a beaker and decomposed with hydrochloric acid in the usual manner. The uric acid was filtered on to a Gooch, well washed, dried and weighed. It was then dissolved in lithia and the solution made up a litre. 100 c.c. of this standard solution was used for standardisation of the permanganate reagent.

FINAL TESTS ON THE METHOD.

The accuracy of the method for estimating uric acid in poultry excreta was submitted to further confirmation by the tests outlined below.

1. A weighed amount of ammonium urate of known uric acid content was submitted to the complete series of processes involved in the method as outlined in the previous section. The following satisfactory result was obtained.

$$\text{Weight of uric acid in ammonium urate} = 0\cdot 1451 \text{ gm.}$$
$$\text{,,} \qquad \text{,,} \qquad \text{recovered} \qquad = 0\cdot 1445$$

2. In order to ascertain definitely whether the presence of protein interfered with the accuracy of the method, the following decisive experiment was carried out. To a weighed amount of ammonium urate was added approximately half its weight of a mixture of equal quantities of gliadine and glutenine. The latter proteins were chosen on account of the probability of their presence in poultry excreta arising from the consumption of grain diets. The following results were obtained in the estimation of uric acid in such a mixture.

$$\text{Weight of uric acid in ammonium urate} = 0\cdot 1845 \text{ gm.}$$
$$\text{,,} \qquad \text{protein mixture added} = 0\cdot 1000$$
$$\text{,,} \qquad \text{uric acid recovered by } (a) \text{ direct weighing} = 0\cdot 1830$$
$$(b) \text{ KMnO}_4 \text{ titration} = 0\cdot 1820$$

It will be noted that the presence of a relatively large proportion of entirely undigested protein does not in any way affect the accuracy with

which the determination is carried out. Nor is this surprising in view of the fact that the material is subjected to the alternate action of hot acid and alkali during the estimation. It was noted that the clear alkaline filtrate obtained after precipitating the ammonium urate from the lithia extract gave a precipitate of protein on acidification. The protein is not to any noticeable extent removed from solution by the action of ammonium chloride, and this is further borne out by the observation that during the decomposition of the ammonium urate with hydrochloric acid, a perfectly water clear solution was at first obtained as was the case when ammonium urate, without any addition of protein, was employed. From this clear solution, uric acid separated in characteristic crystalline form. If, however, the ammonium urate precipitate should be contaminated with a little protein, then the latter would be removed during the treatment with hydrochloric acid.

3. In the concluding trial, four samples of about 10 gm. of well mixed poultry excrement were weighed out into beakers. The uric acid content was determined in two of the samples by the new method. The excellent agreement between the duplicates in this trial will be noted. To the third and fourth samples were added known weights of ammonium urate and the total uric acid now present was determined. Using the figure obtained in duplicates 1 and 2, it was possible to calculate the uric acid content of the samples of excreta weighed out into beakers 3 and 4. These amounts were then subtracted from the total uric acid figures as found by analysis. The resulting figures should agree with the amounts of uric acid originally added in the form of ammonium urate. Considering the severity of the test, the actual agreement obtained was very satisfactory, as is shown by the following figures:

	(1)	(2)
Percentage of uric acid found in excreta ($KMnO_4$ titration)	3·91 %	3·91 %
	(3)	(4)
Weight of uric acid added as ammonium urate	0·1479 gm.	0·1630 gm.
„ „ determined by analysis ...	0·1500	0·1646

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CRITICAL NOTE ON THE METHOD OF CORRECTING PROTEIN DIGESTION COEFFICIENTS.

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THE object of this note is to call into question the soundness of the generally accepted method by which the so-called apparent protein digestion coefficients of foodstuffs are corrected.

The digestion coefficient for the protein of a foodstuff as ordinarily determined by animal experiment does not afford a strictly accurate idea of the actual digestibility of that constituent. The figure represents a minimum value, and this arises from the well-known fact that the faeces do not consist solely of undigested food residues, but are to an appreciable extent contaminated by nitrogenous products which have been secreted into the alimentary tract and have escaped reabsorption. The nature of this so-termed metabolic material is twofold, being partly protein (mucus, epithelium, etc.) and partly non-protein (residues of digestive secretions, etc.). Before proceeding to a critical survey of the correction method, which is designed ostensibly to enable allowance to be made for the presence of such products in the faeces, it is of essential interest to consider briefly the true significance of the term "protein digestibility."

It appears to be a warrantable assumption that, given the optimum conditions, most, if not all, proteins would be fully digestible, *i.e.* possess a digestion coefficient of 100. This remark follows from a consideration of the comparative ease with which the peptide linkage is disrupted during hydrolysis. The question naturally arises as to why this theoretical value is never encountered in animal experiments, even when allowance has been made for the presence of nitrogenous metabolic products in the faeces. Further, what is the explanation which accounts for the wide variation in the digestibility of the proteins of the different foodstuffs? Several reasons may be adduced in answer to these questions.

(1) The protein constituent may be in some measure "protected" from enzymic activity by being embedded in indigestible material such

as cellulose. This consideration probably explains why protein from the concentrates is digested more thoroughly, as a rule, than protein from roughages. The protein from the latter is surrounded by tougher and more fibrous cell walls than that of the concentrates.

(2) The foodstuff may possess a tough or tenacious physical character, which prevents its being readily penetrated and attacked by the digestive fluids. An extreme instance of this is gristle. Such material, unless very thoroughly masticated, may almost wholly escape digestion.

(3) The foodstuff may be consumed in such a form that a proportion of it escapes being reduced to a state of fine division by mastication. An example of this is afforded in the feeding of coarsely ground maize to pigs, when small pieces of grain, apparently unaffected by the digestive processes, find their way into the faeces. Such excreted grain must contain the proteins of maize in an unaltered form. It is also common to find unaltered grain in the excreta of poultry.

(4) The foodstuff may have been submitted to cooking processes which have brought about coagulation of protein. In such circumstances the solution of the protein by digestion will be rendered more difficult and consequently a larger proportion of undigested protein will be recovered in the faeces.

(5) The rate of passage of the foodstuff through the alimentary tract may be such as to allow only of incomplete reaction of the protein with the various proteolytic enzymes. A further reason may be found in the establishment of equilibrium between reacting substances and products of reaction. It is quite conceivable that such an equilibrium characterises the gastric digestion of protein, although it is less likely that such a state of affairs could exist in the final stages of digestion in the small intestine, where the products of the reaction, namely amino acids, are being absorbed continuously from the tract. In this connection, however, it is important to bear in mind that if for any reason any part of the protein escapes breakdown in the peptic and tryptic stages of digestion, then such a fraction will entirely escape digestion, since erepsin appears to be, with one or two exceptions, entirely without· action on unchanged proteins.

With herbivora, it is probable that the different results obtained for the digestibility of the protein from different sources are due mainly to the operation of the first of the factors enumerated above. Thus, when a protein digestion coefficient is given as 70, the figure actually signifies that the protein is so "protected" in the foodstuff that during the passage of the latter through the digestive tract, a portion of the

protein entirely escapes the action of enzymes and this fraction, when added to the nitrogenous substances of the faeces not arising from food-stuff protein, represents 30 per cent. of the consumed protein. The main point is that the protein thus passing into the faeces should not be regarded literally as "indigestible protein" but rather as "undigested protein," since under suitable conditions the undigested fraction would be attacked and hydrolysed by enzymes.

It follows therefore that the proteins of different foodstuffs do not differ so much in regard to actual digestibility, but rather in respect of their accessibility to the digestive enzymes. It is in respect of this latter factor that the digestion trial affords information and it is clear that the important constituent of the faeces in connection with the determination of protein digestibility is the undigested food protein. With this idea in mind, the illogical nature of the usual method of correcting the protein digestion coefficient becomes apparent. The method consists in the treatment of the faeces with pepsin-HCl reagent at 37° C. under specified conditions and the subsequent determination of the amount of nitrogen in the material which remains undissolved in this process. This nitrogen is assumed to constitute a measure of the undigested food protein and the calculation of the protein digestion coefficient is made on this basis. It seems, however, at once obvious that this assumption is of very dubious validity, since the reagent employed in the reaction, namely, pepsin-HCl, is one which is used to effect solution of protein. In view of this, it is difficult to believe that the protein residues of the foodstuff are still present in their entirety in the insoluble material remaining after the action of the pepsin-HCl solvent.

This method of obtaining the true protein digestion coefficient was first used by Pfeiffer (2) and originally encountered considerable opposition. The latter, however, appears to have been forgotten and the method has come to be used without question. Pfeiffer claimed to have proved that the nitrogenous metabolic products of the faeces could be brought completely into solution by treatment with pepsin-HCl according to the conditions laid down by Stutzer (3). In demonstrating this, he used pig faeces which had been derived from a protein-free diet. Paijkull (4), however, showed that a flocculent precipitate separated from a solution of the mucous substance of bile in 0·3 per cent. HCl by treatment with pepsin at 40° C. Bülow (5) pointed out that although Pfeiffer had shown that treatment of pig faeces arising from the consumption of a nitrogen-free diet with pepsin-HCl at 37° C. brought about solution of the whole of the metabolic nitrogen, yet the more important point had not been

proved, namely, that when normal faeces are acted upon by this reagent, *only* the metabolic nitrogen is removed. No proof had been adduced to show that residual food nitrogen would not also be brought into solution by this method.

Pfeiffer, however, argued that if the protein had defied solution in the natural digestive processes, then it would also resist the action of the proteolytic enzyme during the treatment of the faeces with pepsin-HCl. This view, in the writer's opinion, assumes the protein of the faeces to be literally indigestible protein and not merely a fraction which has escaped gastric digestion by reason of the presence of "protective" substances. Subsequent to peptic digestion, the foodstuff has been subjected to the action of enzymes and bacteria which have removed wholly or in part such protective materials as fat and fibre. Furthermore, the faeces have also been dried and finely powdered, so that it is improbable that under these conditions the residual food protein will be inaccessible to the action of pepsin when the sample of faeces is treated with pepsin-HCl. Pfeiffer's method virtually means that the food residues are subjected to a further digestion under most favourable conditions. Consequently a high protein digestion coefficient will be obtained in this way which will not truly reflect the capacity of the animal for digesting the protein in the consumed foodstuff.

In order that this method should be reliable, it is necessary to assume that pepsin-HCl dissolves completely all forms of metabolic nitrogenous material whilst at the same time the residual food protein is entirely unattacked. The reverse, however, is probably true, namely, that the metabolic nitrogenous substances are not wholly dissolved (*e.g.* mucous material, epithelial waste, etc.) whilst appreciable amounts of residual food protein are brought into solution.

The application of the Pfeiffer method, by adding on to the natural digestive process a further proteolytic digestion under favourable conditions, must lead to anomalous results. A case in point may be referred to briefly. Although it is well known that the initial treatment of a foodstuff may affect its digestibility considerably, it is difficult to see how, by the use of the Pfeiffer method, the effect of such treatment on the protein digestibility could be revealed. The corrected protein digestion coefficients for cooked and uncooked maize, for instance, would be identical, since in both cases the value of the coefficient would be controlled by the final digestive action of pepsin-HCl on the residual food protein of the faeces. For the same reason, it would be impossible to investigate the influence of idiosyncracy and species on the digestibility of the protein of a given foodstuff.

The moderate agreement which is sometimes noted between the protein digestion coefficients corrected as above and those obtained by direct action on the foodstuff of pepsin-HCl at 37° C. has been cited as a confirmation of the correctness of Pfeiffer's method. It might be urged that such agreement is not altogether unexpected, since the controlling factor in both cases is the reaction of pepsin-HCl with foodstuff or faeces. In the writer's opinion, however, such agreement when obtained is accidental. Cases exhibiting striking lack of agreement may be quoted. Crowther and Woodman (1), for instance, obtained the value of 90 for the digestion coefficient of palm kernel meal protein as determined by animal experiment and corrected by Pfeiffer's method. By direct treatment of the meal with pepsin-HCl *in vitro* the low value of 78 was obtained. The result appears to confirm the supposition of the writer that although the protein of the foodstuff may be in some degree "protected" from the action of pepsin, yet in the faeces this "protective" action is no longer exerted and consequently the residual food protein of the faeces is readily attacked by pepsin-HCl.

How then may the true digestibility of the protein of a foodstuff be measured? It must be admitted that the estimation of the residual food protein in faeces presents a difficult if not impossible problem. A true protein determination on the faeces would be helpful, if it were not for the presence of protein among the metabolic nitrogenous products. It is probable that the most reliable values are obtained by acting on the foodstuff with pepsin-HCl *in vitro*. The by no means improbable assumption involved in this view is that the fraction of the protein of a foodstuff which escapes the action of pepsin in the stomach of an animal is ultimately excreted in the faeces as undigested protein. The chief objection to this method is its arbitrary nature, the process being carried out under certain definite conditions irrespective of whether the foodstuff is rich or poor in protein. That the results obtained in this manner may sometimes be difficult of interpretation is shown by the following experiments carried out by the writer. Six lots of about 5 gm. of finely ground linseed cake were weighed out into beakers and each sample was treated with pepsin-HCl at 37° C. according to the conditions specified in the method. The undissolved residues were then filtered off on to hardened filter papers and were well washed with hot water. The nitrogen content of two of the residues (*A* and *B*) was then determined, whilst the remaining four residues were washed back into the beakers and were submitted to the action of pepsin-HCl as before. After this treatment, the nitrogen content of the third and fourth residues (*C* and *D*) was determined, whilst the fifth and sixth residues were again washed back into the

beakers and the digestion with pepsin-HCl was carried out a third time. The final residues (E and F) were filtered off, washed and their nitrogen content determined. The following results were obtained:

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Nitrogen in linseed cake = 5·225 %.

Undigested nitrogen after first treatment (mean of A and B) = 1·366 %.

Digestion coefficient of linseed cake protein = 73·8 %.

Undigested nitrogen after second treatment (mean of C and D) = 0·714 %.

Digestion coefficient of linseed cake protein = 86·3 %.

Undigested nitrogen after third treatment (mean of E and F) = 0·647 %.

Digestion coefficient of linseed cake protein = 87·6 %.

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The low result obtained after the first treatment is probably the consequence of the establishment of equilibrium in the reaction between protein and enzyme. The value thus obtained for the protein digestion coefficient can only accurately reflect the extent to which the protein would be digested naturally provided a precisely similar equilibrium is set up in the stomach of the animal. This is scarcely to be anticipated, since the digestion *in vitro* is fundamentally dissimilar from true gastric digestion. It is more probable that the value obtained after the additional treatment with pepsin-HCl more nearly approximates to the digestibility of the protein in the animal.

The foregoing considerations reveal therefore the difficulties attaching to any attempt to determine the exact extent to which the protein of a foodstuff is digested by an animal. On the other hand, there exist cogent reasons why the "apparent" digestion coefficients should be retained. It is these values which have been employed in the calculation of food values according to Kellner's system. The corrected coefficients have not so far been utilised for this or any similar purpose. Furthermore, since the amount of metabolic material in the faeces is actually a crude measure of the difficulty experienced in digesting the food and is related not only to the amount but also to the nature of the organic matter digested, it is not without value to have a rough expression of these factors in the protein digestion coefficient. The corrected coefficient is of merely theoretical interest; from the standpoint of food values, it is of greater moment to possess information concerning the amount of nitrogen lost to the organism through the faeces per 100 gm. of protein consumed.

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STUDIES IN CROP VARIATION.

III. AN EXAMINATION OF THE YIELD OF DRESSED GRAIN FROM HOOS FIELD

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(With Six Text-figures.)

I. INTRODUCTORY.

In 1921, Mr R. A. Fisher published the results of a statistical analysis of the yield of dressed grain from the Broadbalk Wheat field at the Rothamsted Experimental Station (1). The present paper sets forth the results of a similar analysis of the yield of dressed grain from the Hoos Barley field of the same station. Of the 28 plots which are at present in existence, 13 have received the same manurial treatment since 1852, and these were selected for detailed analysis. The examination extends over the 70 years 1852–1921—the last year available when the investigation was started.

Even in the case of the 13 selected plots, certain slight alterations and modifications have taken place from time to time. These are recorded below.

CHANGES IN MANURIAL TREATMENT.

(a) In 1852 the two plots 5–A and 5–O were laid down next to each other; both received 392 lb. superphosphate and 300 lb. sulphate of potash, but in addition 5–A received 100 lb. sulphate of ammonia and 100 lb. ammonium chloride per acre. The following year, plot 5–O was used for a nitrate of soda test, and plot 5–A was divided, one half, still called 5–A, receiving ammonium salts in addition to superphosphate and sulphate of potash; and the other half, in future called 5–O, receiving only the two latter manures. In the analysis, the yield of the original 5–O has been used for 1852. The width of the new plot is only 87 links, and is adjacent to that used in 1852; hence the inherent fertility of the soil was probably about the same. The residual effect of the one year's application of ammonium salts was probably not very great, and would only have an insignificant effect upon the yield of the following year.

(b) In 1858 the quantity of sulphate of potash was reduced from 300 lb. to 200 lb. per acre on plots 3, 4 and 5, series O and A; also, the quantity of sulphate of soda was reduced from 200 lb. to 100 lb. per acre on plots 3 and 4 in series O and A.

(c) In the year 1880, 200 lb. per acre of both sulphate of ammonia and ammonium chloride were applied on plot 5–A, but in 1881 the old quantity of 100 lb. per acre of each was reverted to.

(d) On the A series, in 1887, ammonium chloride was replaced by sulphate of ammonia on the basis that 125 lb. of sulphate of ammonia was equivalent to 100 lb. of ammonium chloride. The latter was used again in the following years.

(e) From 1898 till 1902 inclusive, 400 lb. of basic slag was applied on plots 2, 4 and 5, series O and A, instead of 392 lb. superphosphate.

(f) For the season of 1901, half of each of the plots 1–4, series A, received a dressing of 258 lb. of bicarbonate of ammonia instead of the usual 100 lb. sulphate of ammonia and 100 lb. ammonium chloride. The halves were harvested separately in 1901, but no separate weighings were recorded in the following year, so that the residual effect of the bicarbonate of ammonia cannot be estimated.

(g) The seed for the 1908 crop was treated with Schering's formalin before sowing: every four bushels of seed was soaked in 8 gallons of water in which $\frac{1}{2}$ lb. formalin had been dissolved. After soaking in the solution for 10 minutes, the seed was drained through a brass wire sieve, and spread thinly over the floor until dry.

(h) During the two years 1917 and 1918 sulphate of potash and sulphate of magnesia were omitted from the plots usually in receipt of these manures, viz. plots 3 and 4, series O and A. In 1917 also ammonium chloride was again replaced by sulphate of ammonia on plots 1–5, series A, and has not been used since.

It is difficult to say what would be the effect of these various changes upon the statistical results. The influence (if any) of such temporary alterations as those of (c), (d) and (g) would doubtless appear as annual variation. The influence of substitutions such as that of (e) is more difficult to determine. If we may assume that the manure actually applied was equivalent to that usually used—and the records infer that such was the intention of Sir John Lawes—the modification would have no influence upon the variation. For 1901 the yield upon the half plot which received the usual dressing of sulphate of ammonia and ammonium chloride has been used in the present analysis. As no distinction was made between the two sections in the records of 1902, a general figure

for the whole plot had to be used for the plots affected. If the residual effect of bicarbonate of ammonia is the same as that of the salts which it replaced, this alteration would have no effect upon the statistical analysis. On plot 4–A, the plot which was most affected by the substitution, the yield on the half plot receiving bicarbonate of ammonia was 18 bushels per acre in 1901 compared with 24 bushels on the other half, a difference of 6 bushels. The difference in the yield on the two halves in the following year was probably negligible, therefore—at least as regards the present investigation.

The Fallow of 1912.

Owing to the growing foulness of the plots, the whole field was fallowed in 1912. This made a rather unfortunate break in an otherwise uniquely continuous series. To fill this gap for the purpose of statistical analysis the average of the six years 1909–11 and 1913–15 was adopted as an estimated yield for 1912. The crop for 1913 was much heavier than usual, of course, on account of the preceding fallow, and for this reason it might have been better if this year had not entered into the estimate for 1912. On the other hand a yield as high as—in some cases even higher than—that of 1913 was obtained from the majority of plots in 1916. Thus the average figure adopted was considered appropriate for the purpose.

Changes in Variety.

From time to time the variety of seed used has been altered. Below is given a list of the varieties used during the 70 years under consideration:

1852–1880	Chevalier Barley
1881–1890	Archer's Stiff Straw Barley
1891–1897	Carter's Paris Prize
1898–1901	Archer's Stiff Straw Barley
1902–1905	Hallett's Pedigree Chevalier Barley
1906–1916	Archer's Stiff Straw Barley
1917 onwards	Plumage Archer.

Such changes give rise to a certain amount of variation due to genetical differences. It will be noticed that since 1880 there has been a tendency to change the variety of seed used at fairly frequent intervals. The more frequent the changes in variety, the greater the likelihood that the genetical effects will be included in the annual causes of variation. If the variety were altered at infrequent intervals one would expect the genetic influences to appear as slow changes. A study of the polynomial curves provides good evidence that the effect of genetic differences is by no means an important cause of the slow changes revealed.

NUMBER OF ROWS OF BARLEY.

The number of rows of barley on each plot has varied somewhat during the period. In 1852 it is recorded that there were "20 rows upon each land" and that sowing commenced "on the 19th land from the Clover Experimental plots." The meaning of the term "land" is obscure, but it appears to have been a rather indefinite measure—the men "stepped it out." If the drilling of the seed commenced at the edge of the barley plots, on plot 7, as one may reasonably suppose, then a "land" would be approximately 16 feet. Taking into consideration the quantity of seed used and the width of the plots, and assuming a land to be 16 feet, it is estimated that there were probably 73 rows per plot in 1852. No further information is given concerning the number of rows until 1897 when it is recorded that there were 78 rows per plot. At the present time there are 90 rows. The exact date of the change from 78 to 90 is unknown, but it appears to have taken place prior to 1911. Or it may be that the number of rows was increased to 90 many years earlier and that the note in 1897 only records a deviation from the usual procedure for that year. The quantity of seed used per acre remained the same throughout the whole period of 70 years ($2\frac{1}{2}$ to $2\frac{3}{4}$ bushels per acre). It is probable therefore that the variation in the number of rows was the result of variations in the distribution of the seed, designed to test the relative advantage of thick and thin sowing in the rows. Owing to the great annual variation due to weather conditions the effect of these changes in the number of rows is obscured.

OBJECT OF PAPER.

The object of the present paper is to determine the slow changes which have taken place in the mean yield of the thirteen selected plots; and to indicate the relationships between manurial treatment and mean yield and deterioration respectively. No attempt is made in this present paper to examine the effect of rainfall upon the barley crop. Methods have recently been devised by Mr R. A. Fisher which enable one to make a more detailed investigation of this problem than has hitherto been possible. The amount of time and labour involved in an adequate study of this interesting subject of the relationship between weather and crops, by the methods recently elaborated, made it appear desirable to leave this question for future study.

METHOD OF ANALYSIS.

The method developed by Mr R. A. Fisher in connection with his examination of the yield of dressed grain from Broadbalk [1] has been used in the following investigation. This involves fitting polynomials to the various series of plot yields, and analysing the total variance into the amount due to deterioration, to slow changes and to annual causes.

II. MEAN YIELDS IN RELATION TO MANURIAL TREATMENT.

Of the 26 plots considered in this section, half have received the same manurial treatment for a shorter period than 70 years.

From 1917–20 inclusive, rape cake was unobtainable, and the omission of this important source of nitrogen was very evident in the rapid fall in the yields from the four plots in the "C," or Rape Cake series. The mean yields for these plots have been calculated upon the 64 years 1852–1916 (1912 being fallowed).

The "AA" series originally received a double dressing of ammonium salts 1852–1858, and then single ammonium salts until 1867, since which time the nitrogenous manure has been supplied in the form of an equivalent amount of nitrate of soda. In this series, therefore, the mean yields have been based upon the 53 years 1868–1921.

The "AAS" series received the same treatment as the "AA" series until 1864, when 200 lb. silicate of soda and 200 lb. silicate of lime were added to the previous manure. The change in the nitrogenous manure made in 1868 applied to both the "AA" and the "AAS" series, and at the same time, 200 lb. silicate of soda replaced the 200 lb. silicate of lime previously used. Consequently the mean yields given in Table I have been based upon the years 1868–1921 only.

Plot 7–1 was originally part of the Farmyard Manure plot, and received the usual dressing of 14 tons dung per acre from 1852 till 1872. In that year the plot was halved, and the portion now known as 7–1 was left unmanured, and has remained so for the last 50 years. The mean yield of this plot, therefore, is based upon the 49 years 1872–1921.

The standard manuring and the mean yields of each of the 26 plots are given in Table I.

In order that comparisons may more easily be made between plots in different series, averages for the years common to all, viz. 1868–1916, are also given.

Plot 7–2 has a mean yield which is significantly higher than that of any of the others. This is no doubt due to the fact that the farmyard

manure supplies approximately twice as much nitrogen per acre as the other sources of supply.

Table I.

Manures per acre

Plot	Farmyard manure tons	Rape cake lbs.	Ammonium salts lbs.	Nitrate of soda lbs.	Super-phosphate cwt.	Sulphate of potash lbs.	Sulphate of soda lbs.	Sulphate of magnesia lbs.	Silicate of soda lbs.	Mean yield 1868-1916 bushels	Mean yield per acre and standard error of mean bushels
1-O	—	—	—	—	—	—	—	—	—	12·13	13·97 ± ·76
2-O	—	—	—	—	3·5	—	—	—	—	17·49	19·62 ± ·91
3-O	—	—	—	—	—	200	100	100	—	12·75	15·00 ± ·87
4-O	—	—	—	—	3·5	200	100	100	—	17·16	19·85±1·06
5-O	—	—	—	—	3·5	200	—	—	—	14·24	16·22 ± ·97
1-A	—	—	200	—	—	—	—	—	—	23·06	24·90±1·10
2-A	—	—	200	—	3·5	—	—	—	—	34·79	37·11±1·51
3-A	—	—	200	—	—	200	100	100	—	25·47	27·13±1·15
4-A	—	—	200	—	3·5	200	100	100	—	39·79	40·75±1·14
5-A	—	—	200	—	3·5	200	—	—	—	32·64	34·92±1·22
1-AA	—	—	—	275	—	—	—	—	—	26·39	25·52±1·14
2-AA	—	—	—	275	3·5	—	—	—	—	40·75	40·12±1·24
3-AA	—	—	—	275	—	200	100	100	—	27·30	26·14±1·12
4-AA	—	—	—	275	3·5	200	100	100	—	40·10	39·31±1·22
1-AAS	—	—	—	275	—	—	—	—	400	32·81	31·79±1·17
2-AAS	—	—	—	275	3·5	—	—	—	400	41·92	41·15±1·33
3-AAS	—	—	—	275	—	200	100	100	400	34·62	33·16±1·28
4-AAS	—	—	—	275	3·5	200	100	100	400	43·04	41·63±1·27
1-C	—	1000	—	—	—	—	—	—	—	35·74	38·38±1·14
2-C	—	1000	—	—	3·5	—	—	—	—	38·05	40·56±1·18
3-C	—	1000	—	—	—	200	100	100	—	34·54	37·00±1·20
4-C	—	1000	—	—	3·5	200	100	100	—	38·11	40·57±1·21
6-1	—	—	—	—	—	—	—	—	—	13·23	15·51 ± ·85
6-2			20 bushels ashes from laboratory furnace							14·83	16·46 ± ·83
7-1	—	—	—	—	—	—	—	—	—	25·01*	22·46±1·56
7-2	14	—	—	—	—	—	—	—	—	46·18	46·17±1·20

* 1872—1916 only.

The great importance of phosphoric acid is brought out very clearly by a comparison of the mean yields of plots 3 and 4 in each series. The manurial treatment of these plots differs only in respect of the omission (plots 3) or inclusion (plots 4) of this source of nutriment. In every case the mean yield of plot 4 is significantly greater than that of plot 3.

Unfortunately there are no Broadbalk plots which exactly correspond with any of the plots 3 and 4 on Hoos Field. The only two wheat plots which can be used to judge the effect of superphosphate are plots 10 and 11. Both of these plots are starved for potash and consequently a comparison of their means over the whole period is hardly a just one, since the later yields are greatly influenced by potash starvation. That superphosphate is of more importance to the Barley crop than to the Wheat crop may be seen by comparing the mean yields of plots 10 and 11 Broadbalk 1852–1870, before the lack of potash was so acute, and the means for the same period of the two Hoos Field plots 1–A and 2–A,

which most resemble the wheat plots in manurial treatment, being also starved for potash.

Table II.

	Wheat plots				Mean yield per acre 1852–1870
Plot 10	Double ammonium salts	...	...	...	24·93±1·69 bushels
„ 11	„	„	„	and superphosphate	28·98±1·78 „
	Barley plots				
Plot 1–A	Single ammonium salts	...	...	...	32·25±1·93 bushels
„ 2–A	„	„	„	and superphosphate	47·17±1·81 „

The same remarkable response of barley to superphosphate is shown by plots 1 and 2 in series O and AA and AAS. Only in the rape cake series does the addition of superphosphate fail to produce so marked an increase in mean yield. This is doubtless due to the fact that rape cake contains phosphoric acid, so that all plots receive a small quantity of it whether given specifically in the form of superphosphate or not. It is estimated that about 4 per cent. of rape cake is phosphoric acid, so that the "C" series receives per acre an extra 40 lb. of this important nutrient. The usual superphosphate dressing is 392 lb. per acre containing 35 per cent. phosphoric acid. The 1000 lb. rape cake per acre therefore, in addition to giving 49 lb. of nitrogen, is equivalent to an extra 114 lb. superphosphate. In the light of this fact, the relatively unimportant increase in mean yield of those plots receiving extra phosphoric acid over those receiving only the quantity contained in the nitrogenous manure, suggests that the rape cake series forms a partial illustration of the "law of proportional returns." From the last column of Table I we see that the additional 128 lb. phosphoric acid (given as 392 lb. superphosphate) produces an increase of only 2·31 bushels in the absence of alkali salts and 3·57 bushels when these salts are present. The corresponding average increases given by the same quantity of superphosphate in the series A and AA is 13·05 and 13·56 bushels respectively. If we take the average of the excess of 1–C over 1–AA and 1–A as an estimate of the increase in yield due to the phosphoric acid contained in the rape cake, we find that the first "dose," equivalent to 114 lb. superphosphate, gives an increase of 11 bushels per acre compared with an increase of approximately 3 bushels from the extra 392 lb. Thus the greater quantity of phosphoric acid yields a less proportionate return than the smaller. The information is insufficient to determine what quantity, other conditions remaining unchanged, would yield the greatest proportional return.

A comparison of the plots 2–O with 5–O and 2–A with 5–A reveals a startling and unexpected effect from sulphate of potash: in both series the plot without has done better than the plot with potash. The lack of response to this important mineral is surprising, and the only explanation that has been brought forward in the past is the fact that the plots 5–O and 5–A lie at the west end of the experimental land, while the other plots in the two series lie at the east end. This might influence the yield from the potash plots in two ways:

1. The natural fertility of the soil at the west end might be considerably lower than that at the east end.

2. The plots at the west end are to some extent affected by the shade of trees, and this might account for the low yield.

PLAN OF HOOS FIELD

PERMANENT BARLEY PLOTS.
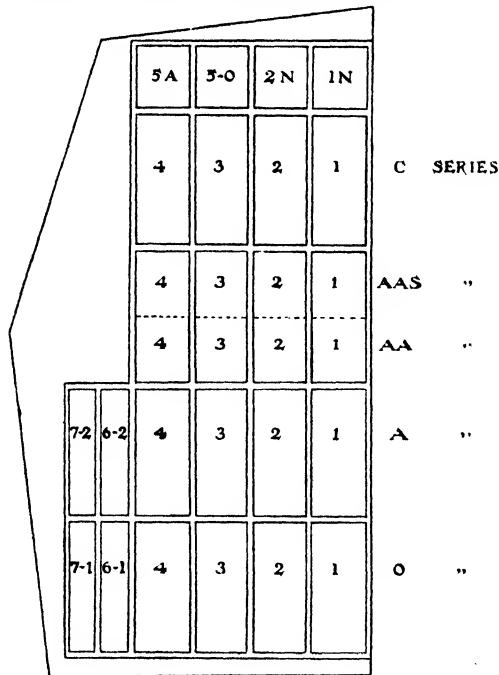
No data exist by which the uniformity of the field at the beginning of the experiment can be determined. There is a certain amount of evidence, however, against the view that position in the field accounts for the low yields on the potash plots.

Plot 1–N lies at the west end of the field, as do plots 5–O and 5–A (see plan). This plot has received a single dressing of nitrate of soda since 1853. Plot 1–AA received double ammonium salts 1852–1858, and single ammonium salts until 1867, since when it has received a single dressing of nitrate of soda. This plot lies a little further west than the "A" series. If the fertility of the field decreases from east to west we should expect the average yield from plot 1–N from 1868–1921 to be significantly lower than that from plot 1–AA for the same series of years. Actually the yield on plot 1–AA is significantly lower than that of plot 1–N: the yields being 25·52 and 28·00 bushels per acre respectively, a difference of 2·48 bushels. The standard error of the difference is ± ·50 so that a difference of more than one bushel would be significant. It may be the lower yield on the "AA" plot is the result of the effect of previous manurial treatment upon the texture of the soil. Or it might be urged that the central part of the field in which the "AA" plots lie, is less fertile than either end. During the decade 1858–67 the AA and the A series had the same treatment, viz. single ammonium salts, and for this period the "AA" plots show no sign of lower fertility, as the following figures show.

Plot	A series. Bushels per acre	AA series. Bushels per acre
1	30·48	33·65
2	49·62	50·24
3	33·37	36·06
4	48·05	50·38

Hence there seems no basis for assuming lower fertility at the west end of the field.

There is also a small amount of evidence which can be cited against the view that the shade of trees accounts for the low yield on plots 5–O and 5–A. From 1855 till 1892, the land lying immediately west of the plots in question was used for experimental purposes. The plot, known as "M," was manured with superphosphate, sulphate of soda, and sulphate of magnesia, and may be compared with the O series since it had no nitrogenous manure. Also "M" lies nearer the hedge than even 5–O and 5–A and is more under the shade of the trees. One would expect that the plot receiving potash and superphosphate would give a higher yield than that receiving only superphosphate and sulphates of soda and magnesia.

As shown in Table III, plot 5–O has a lower mean yield than has M, although the latter lies more under the shade of the trees. The difference between the yields of these plots, 1·24 bushels, is approximately twice

its standard error, ·68 bushel, calculated from the yearly differences. It may not be significant, therefore, but it is great enough to show that the unexpected lack of response to potash revealed by the mean yield is due to something more than the shade of trees.

Table III.

Plot	Manuring	Mean yield, 1855–92. Bushels per acre
2–O	Superphosphate only	20·99 ± ·98
4–O	Complete minerals	21·61 ± 1·25
5–O	Superphosphate, sulphate of potash	18·43 ± 1·04
"M"	Superphosphate, sulphate of soda, sulphate of magnesium	19·67 ± ·90

The difference in yield between plot 2–O and 5–O, and that between plot 2–A and 5–A is very significant in both cases if we use as the criterion twice the standard error calculated from the yearly differences.

Table IV.

Plot	Mean yield per acre. Bushels	Difference. Bushels	Standard error of difference. Bushels
2–O 5–O	19·62 16·22	3·40	± ·44
2–A 5–A	37·11 34·92	2·19	± ·74

This apparently harmful effect of potash is difficult to explain. At present we do not know whether the depressed yield is a direct effect of potash upon the barley plant, or whether it is the result of a chemical reaction between sulphate of potash and an unknown element in the soil which is important for the life of the barley plant. It is known that potash tends to retard the processes of development, and that with barley, a spring sown crop, the processes need to be hastened. It may be therefore, that the decreased yield in the presence of sulphate of potash is due to the immaturity of the crop caused by the retardation of development. In the case of the winter wheat of Broadbalk this effect would not be so marked as the crop has a longer time in which to come to maturity. It is worth noting that the results of the wheat experiments on Broadbalk field show a significant increase when sulphate of potash is included in the artificial manure, viz.:

Plot		Mean yield per acre bushels
11	Double ammonium salts and superphosphate	22·05 ± ·91
13	,, ,, and sulphate of potash	30·21 ± ·91

It is interesting to note the effect of the alkali salts—sulphate of potash, sulphate of soda and sulphate of magnesium—by comparing the

yields of plots 1 and 3 in each series, and plots 2 and 4 in each series. The former comparison shows the effect of the three sulphates in the absence of superphosphate, and the latter in the presence of superphosphate.

Table V.

Effect of alkali salts in the absence of superphosphate

Plot	Mean yield. Bushels per acre	Difference. Bushels per acre	Standard error of difference
1–O	13·97		
3–O	15·00	+1·03	±·27
1–A	24·90		
3–A	27·13	+2·17	±·42
1–AA	25·52		
3–AA	26·14	+0·62	±·40
1–AAS	31·79		
3–AAS	33·16	+1·37	±·39
1–C	38·38		
3–C	37·00	−1·38	±·33

Effect of alkali salts in the presence of superphosphate

Plot	Mean yield. Bushels per acre	Difference. Bushels per acre	Standard error of difference
2–O	19·62		
4–O	19·85	+0·23	±·31
2–A	37·11		
4–A	40·75	+3·64 [+·913]	±·82 [±·65]*
2–AA	40·12		
4–AA	39·31	−0·81	±·49
2–AAS	41·15		
4–AAS	41·63	+0·48	±·57
2–C	40·56		
4–C	40·57	+0·01	±·38

* Figures in square brackets refer to years 1852–90 only.

The standard errors here given are calculated direct from the yearly differences, and not from the standard errors of the means given in Table I. The only case where the alkali salts show a significant increase in the presence of superphosphate is in the case of the ammonium salts series. Plot 2–A has shown a marked falling off in recent years, which has always been ascribed to potash starvation. This point is dealt with in Section IV. If we consider the years 1852–90 only we find that the difference in yield between 2–A and 4–A is also insignificant. In the absence of superphosphate, a significant increase is shown by the plots receiving the three alkali salts in the series O and AAS. The rape cake series shows an unexpected significant decrease. Since rape cake contains a certain amount of phosphoric acid one would have expected the effect shown by plots 1–C and 3–C to tend to correspond with that shown by plots 2 and 4 in the other series, but the significant negative effect is difficult to explain. Rape cake also contains about 4 per cent. potash, and the lower yield on plot 3 compared with plot 1 may be more evidence

of the fact that barley needs only a small quantity of potash for its growth, and any excess tends to exercise a harmful influence on the crop.

For the early years, the No Nitrogen series supplies us with a little information with regard to the effect of sulphate of soda and sulphate of magnesia in the absence of sulphate of potash.

Table VI.

Plot	Manuring	Decrease or increase of mean yield 1855–92 over mean yield of Plot 2-0 bushels	Standard error of difference bushels
2–O	Superphosphate	—	—
4–O	Superphosphate and alkali salts ...	+0·61	±·37
5–O	Superphosphate and sulphate of potash	−2·06	±·54
"M"	Superphosphate, sulphate of soda and sulphate of magnesia	−1·32	±·48

These figures indicate that either sulphate of potash alone or sulphates of soda and magnesia alone have a significantly adverse effect on the yield, while together they have but little influence in the presence of superphosphate.

The analysis of the Broadbalk data showed that in the case of wheat soda and magnesia are of little use in the presence of sulphate of potash. A comparison of the plots 4–O and 4–A with their corresponding plots 5–O and 5–A show that in the case of barley these two manures have a significant beneficial effect upon the yield when potassium sulphate is present.

Table VII.

Field	Plots	Other manuring	Mean yield in bushels per acre	
			With sulphate of soda and sulphate of magnesia	Without sulphate of soda and sulphate of magnesia
Broadbalk	7 and 13	Double ammonium salts, superphosphate, sulphate of potash	31·37 ± ·90	30·21 ± ·91
Hoos field	4–A and 5–A	Single ammonium salts, superphosphate, sulphate of potash	40·75 ± 1·14	34·92 ± 1·22
Hoos field	4–0 and 5–0	Superphosphate, sulphate of potash	19·85 ± 1·06	16·22 ± ·97

The superiority of rape cake over ammonium salts and nitrate of soda as the source of nitrogen is seen from a comparison of the average yields given in Table I. This superiority may be ascribed to two causes:

(1) Rape cake supplies the plots with 49 lb. nitrogen per acre, whereas the other two sources of supply are only equivalent to 43 lb. per acre.

(2) As has been pointed out earlier in this section, rape cake contains a certain amount of phosphoric acid, which, as we have seen, is important for the barley crop.

Comparing the mean yields 1868–1921 of the four plots in series A with the corresponding plots in series AA, we see that in all cases except where complete minerals are given, the plots receiving nitrate of soda are significantly greater than those receiving ammonium salts.

In the case of plot 4 the difference is insignificant, but even here the plot receiving nitrate of soda indicates the superiority of this form for the nitrogenous manure.

Table VIII.

Plot	Mean yield in bushels per acre		Difference in bushels AA—A	Standard error of difference
	A series	AA series		
1	22·33	25·52	3·19	±·45
2	33·94	40·12	6·18	±·64
3	24·57	26·14	1·57	±·43
4	38·76	39·31	0·55	±·59

The effect of silicate of soda, when given in addition to nitrate of soda, may be seen by comparing the AA series with the AAS series. Table I shows that, in the absence of superphosphate (plots 1 and 3) the addition of silicate results in a greatly increased yield. The increase in the case of the plots which are in receipt of superphosphate (plots 2 and 4) is not nearly so striking. In both cases, the difference in mean yield between the two series is significant if we use as the criterion the standard error calculated from the yearly differences, viz. ± ·49 bushel. The beneficial effect of silicate of soda is possibly due to the fact that the silicate releases phosphoric acid in the soil, and this would explain the greater increase in the case of the plot which receives no superphosphate.

III. VARIABILITY AND ITS CAUSES.

Of the plots considered in the previous section, only the thirteen which have received the same manurial treatment for the whole period of 70 years, 1852–1921, have been analysed in detail. With these 13 plots it has been possible to distinguish the same three types of variation in the yield of barley as in that of wheat (1), viz.: (a) annual variation; (b) steady diminution due to deterioration of the soil; (c) slow changes other than deterioration. The causes of the first two types of variation have been explained by Mr R. A. Fisher, as follows (1): "The annual variations may be ascribed primarily to the weather, including in that term not only the direct effects of meteorological conditions in stimulating plant growth but also physical effects wrought upon the soil, such as the washing out of plant nutrients and the indirect effects of light, temperature and moisture in stimulating or retarding the increase of bacteria, protozoa

and of the fungal and algal flora of the soil, all of which may be supposed to adjust their activities rapidly to the meteorological conditions. The steady diminution of yield may be unhesitatingly ascribed to deterioration of the soil; either to the exhaustion of natural supplies of potash and phosphorus, or perhaps to that of unknown substances required in small quantities and not supplied in the artificial manure, or to physical changes as yet but little understood or to the gradual exhaustion of the power of the soil in producing nitrates in the soil moisture."

The importance of the slow changes varies considerably and their cause is far more difficult to determine. It is impossible to suggest a cause of the slow changes which will be true for all plots and all crops. It is often necessary to bring much external evidence to bear upon the question, and the possible causes need to be carefully considered for each plot under examination. The slow changes on the Hoos Field barley plots are considered in Section V.

The total variance of the 13 plots, and the amount due to each of the three main causes, are given below:

Table IX.

Plot	Annual variance	Deterioration	Slow changes other than deterioration	Total variance	P for slow changes
1–O	18·86	15·02	6·40	40·28	·00023
2–O	37·80	9·87	10·61	58·28	·0013
3–O	23·33	19·03	10·30	52·66	·000011
4–O	46·25	15·90	16·03	78·18	·00019
5–O	44·70	16·54	4·87	66·11	·138
1–A	47·69	27·37	9·26	84·32	·015
2–A	104·81	50·91	3·33	159·05	·725
3–A	52·87	34·47	5·47	92·81	·159
4–A	74·08	15·01	2·31	91·40	·736
5–A	57·11	36·25	10·91	104·27	·00046
6–1	21·38	19·82	8·95	50·15	·000023
6–2	31·24	11·96	5·32	48·52	·028
7–2	83·68	7·98	9·60	101:26	·064

The deterioration is here represented by a linear function. It is probably more truly represented by an exponential curve, for one would expect the rate of deterioration to decrease as the reserve supplies of plant nutrients, stored in the soil, become exhausted and thereby reduce the power of the soil to support as heavy a crop as previously. The influence of slow changes other than deterioration, however, makes it difficult to distinguish more than the linear effect of the exhaustion of soil nutrients.

The annual variance accounts for a greater proportion of the total variance than either of the other two causes, while the slow changes other than deterioration are relatively unimportant. This is seen more clearly in Fig. 1 which shows the relative variance $\left(\dfrac{100 \,.\, \text{actual variance}}{(\text{mean})^2} \right)$ of the 13 plots, the plots being placed in order of mean yield.
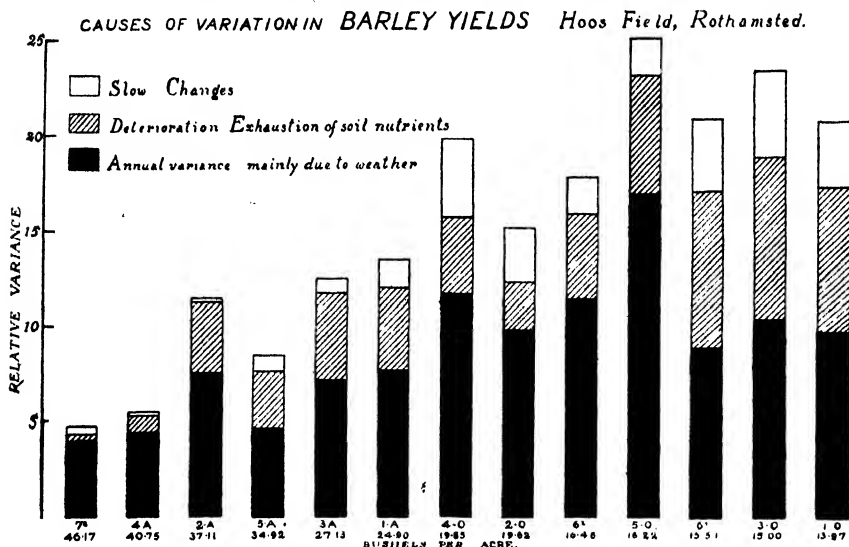
Fig. 1.

The farmyard manure plot (7–2) not only has the greatest mean yield, but the least relative variance. The plot receiving "complete artificials" is not much more variable than 7–2, the difference being accounted for by the greater susceptibility of the artificials to meteorological conditions, and by greater deterioration due possibly to the exhaustion of unknown but important chemicals not supplied with the artificial manure.

The greater relative variance of those plots which receive no nitrogenous manure is very obvious. This is accounted for to a considerable extent by the increased effect of weather conditions, probably the washing out of the natural nitrogen supply during winter. With the "A" series the ammonium salts are not put on until the spring, when the plant is ready to make use of the nitrogen, and hence not as much is lost. Plot 7–2 is least affected by the weather, showing that with farmyard manure the plot tends to suffer less than the others in a bad season but reaps less benefit from favourable meteorological conditions.

In the "A" series, it is noticeable that the two plots which receive both superphosphate and sulphate of potash (plots 4–A and 5–A) are decidedly less variable than the other three plots which lack either one or both these manures. Such a clear division does not exist in the "O" series, however, though there is a tendency for the reverse relationship to hold. Plot 5–O (superphosphate and sulphate of potash only) is the most variable of all plots, while 4–O (complete mineral manure) is more variable than 2–O (superphosphate only) and is nearly as variable as the other two plots in the series.

The Hoos Field barley plots are, on the whole, much more variable than the Broadbalk wheat plots. This is illustrated in Fig. 2 where certain Hoos Field plots are compared with the Broadbalk plots which receive exactly the same manurial treatment.

RELATIVE VARIANCE OF WHEAT AND BARLEY.
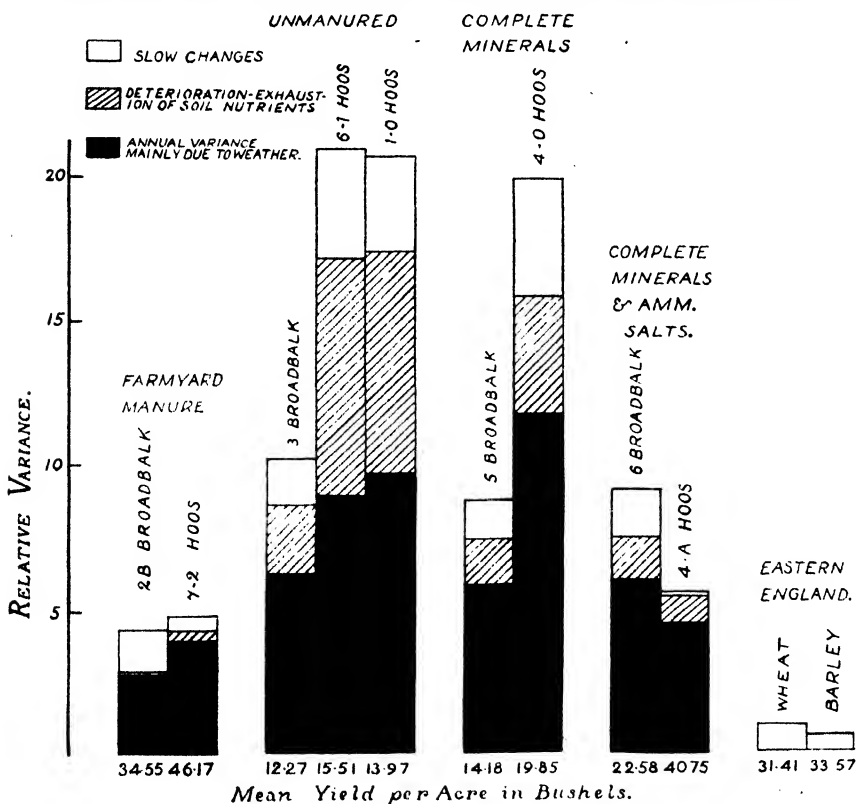
Fig. 2.

Even the farmyard manure plot in the barley series is more variable than in the wheat series, being especially more subject to annual variation. The greater variability of the barley plots is adequately shown in the unmanured and complete mineral plots. Plots 6 and 4–A (complete minerals and single ammonium salts) form the one exception, the barley plot 4–A being considerably less variable than the corresponding wheat plot.

This greater variability of barley as compared with that of wheat is in strange contrast to the result of Mr R. H. Hooker's investigation (2). He used the standard deviation as a measure of variance, and found that in eastern England, in Scotland and in France, barley is less variable than wheat. The different results may possibly be accounted for by the fact that Mr Hooker's investigation was based upon only 21 years, while the present enquiry is based upon 70. It will be seen from Fig. 3 that Mr Hooker finds both wheat and barley in eastern England to be far less variable than is the case with the Rothamsted plots.

This may perhaps be accounted for by the fact that Mr Hooker used an average figure for eastern England, which would include yields from different types of soils. The annual variance of such an average might easily be less than that of a single field since there would probably be compensating increases and decreases over the area included.

IV. ANNUAL DIMINUTION AND DETERIORATION.

The average annual diminution in bushels per acre of each of the selected plots, together with the relative deterioration, are given in Table X. For purposes of comparison the relative deterioration of the corresponding Broadbalk plots is also given.

Table X.

Plot	Mean yield. Bushels per acre	Mean annual diminution. Bushels per acre	Mean annual diminution %	P for deterioration	Relative deterioration	Relative deterioration Broadbalk plots
1–0	13·97	·192	1·37	·000,000	7·69	2·358
2–0	19·62	·155	0·79	·000,044	2·56	—
3–0	15·00	·216	1·44	·000,000	8·45	—
4–0	19·85	·197	0·99	·000,002	4·04	1·502
5–0	16·22	·201	1·24	·000,001	6·29	—
1–A	24·90	·259	1·04	·000,000	4·41	—
2–A	37·11	·353	0·95	·000,000	3·70	—
3–A	27·13	·291	1·07	·000,000	4·68	—
4–A	40·75	·192	0·47	·000,32	0·90	1·469
5–A	34·92	·298	0·85	·000,000	2·97	—
6–1	15·51	·197	1·27	·000,000	8·24	2·358
6–2	16·46	·171	1·04	·000,001	4·42	—
7–2	46·17	·140	0·30	·013,5	0·37	·031

In column 4 "P" stands for the probability that a larger annual diminution would occur by chance owing to the later seasons happening to be, on the average, less favourable than the earlier. This assumes that there has been no real deterioration in the average weather during the 70 years considered. An examination of the rainfall data at Rothamsted shows that there has been no significant change in the total monthly rainfall of any month except December. Mr R. H. Hooker [2] has found that the rainfall in this month has practically no influence upon the barley crop, and therefore any increase in the average rainfall that may have taken place is not likely to vitiate the calculation of P. A more detailed analysis of the Rothamsted rainfall data based upon a six-day period, shows that there has been no progressive change in the total amount of rain falling in any one year. The analysis does indicate, however, that there has been a tendency for the distribution throughout the year to change, the rain tending to fall more in summer and winter and less in spring and autumn than was formerly the case. Mr R. H. Hooker [2] has pointed out that the rainfall in Spring has more influence upon the barley crop than at any other time during the year. This indeed is in accordance with the well-known fact that barley needs to be sown in a fine tilth—a condition which is more likely to be obtained after a period of dry and frosty weather than after a rainy period, since the rain clogs the soil. The evidence seems to indicate, therefore, that if anything the weather conditions for the barley crop have tended to be more favourable, on the average, during the later seasons than the earlier. The calculation of P further assumes that the methods of cultivation have remained equally effective throughout the period.

The deterioration is significant in all cases—even in the case of plot 7–2, where one would only get so great deterioration by chance once in 74 trials. This heavy deterioration on the farmyard manure plot is rather unexpected, for on the corresponding wheat plot the mean annual diminution was found to be insignificant. It is difficult to believe that so great a deterioration is due entirely to the exhaustion of soil nutrients. It may be that an essential chemical food present to some extent in the soil is altogether missing from even farmyard manure; or else is present in such minute quantities as to be entirely inadequate for the needs of the heavy crop, which is annually removed from the plot, so that the marginal supply in the soil is becoming exhausted. Such an explanation is difficult to accept, however, since farmyard manure is so rich in organic matter. On the other hand it may be that the convention ascribing to deterioration the changes represented by the linear term of the fitting

polynomial is not as true in this case as in others. The linear term may partly represent slow changes due to causes other than deterioration.

The much greater relative deterioration of the barley plots compared with the parallel wheat plots is striking. The one exception is the plot receiving complete minerals and a single dressing of ammonium salts as was pointed out in an earlier section. The same phenomenon is revealed if we compare the mean annual percentage diminution of the plots in the two fields. Since wheat requires more nitrogen for its growth than does barley, a truer comparison might be obtained by comparing plot 4–A with plot 7 (double ammonium salts) instead of plot 6 (single ammonium salts). The main facts remain the same, however, except that the mean annual decrement per cent. of plot 7 is the same as for plot 4–A (viz. ·46 and ·47 respectively) while that for plot 6 is rather higher (viz. ·62).

Plot 2–A, superphosphate and ammonium salts, has the greatest annual diminution. During the past decade, the foliage in the centre of this plot has in various years turned yellow quite early in the season. Hitherto this has always been ascribed to potash starvation: it being thought that the heavy crop taken off the plot in the early years had exhausted the potash in the soil more rapidly than the smaller crops of plot 1–A. If the recent fall in the yield of plot 2–A is due to potash starvation one would expect to find indications of a similar effect in other plots starved for potash: for example to find 2–O deteriorating more rapidly than 5–O. But this is not the case; the plot with potash has a greater relative deterioration and a greater mean annual diminution than that without, although the latter has always had the heavier crop and therefore would be exhausting the potash supply more rapidly than the former. It is true, as pointed out earlier, that plots 5–O and 5–A lie in a different part of the experimental land from the rest of their series, and are to some extent affected by the shade of the hedge and trees. But in section II it was shown that the evidence available does not support the view that these factors are responsible for the lower mean yield of the potash plots.

Figure 3 shows that during the years in which the produce of plot M was recorded the plot with potash (5–O) tended to deteriorate at a greater rate than M which received sulphate of soda and sulphate of magnesia instead of sulphate of potash. It has been suggested that there may possibly be present in the soil an essential chemical which sulphate of potash makes more soluble and which is consequently washed out by rain more rapidly and lost to the plant. Hence it would seem desirable

that the cause of the recent fall in the mean yield of plot 2–A and the yellow colouring of the leaves should be thoroughly investigated before being definitely ascribed to potash starvation.
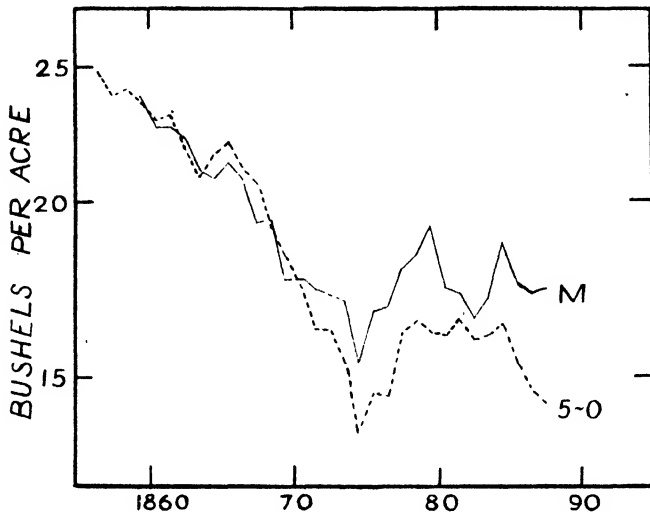
Fig. 3.

In both the "O" and the "A" series, plot 3 shows a rather greater annual diminution and relative deterioration than plot 1. None of these plots receive superphosphate, and the heavier deterioration on plot 3 is doubtless accounted for by more rapid exhaustion of the natural supplies of phosphoric acid, due to the greater yields which have been removed from these plots as compared with the less well manured plots 1.

The greater deterioration of plot 4–O compared with plot 2–O may probably be explained in a similar way, for the yield from the former was higher than that from the latter in the earlier years, and hence may have suffered more acutely from the lack of nitrogen than the plot with the lower yield.

The importance of a good supply of phosphoric acid is seen in the more rapid deterioration of those plots (3–O and 3–A) which are starved in this particular source of nutriment compared with those plots (4–O and 4–A) which have a good supply renewed each year. This fact is greatly emphasised if we consider the mean annual percentage decrements, for, with the exception of 5–O all plots receiving superphosphate have a smaller mean annual decrement per cent. than the remaining plots.

V. SLOW CHANGES IN MEAN YIELD OTHER THAN DETERIORATION.

The course of the slow changes in mean yield is by no means the same for all the plots examined. Groups of similar curves may be differentiated by an examination of the significance of the different terms of the fitting polynomial.

Table XI. Coefficients of the Polynomials.

Plot	B	C	D	E	F
			·000	·000	·0000
1–O	− ·192	·0058	− 110	005	− 005
2–O	− ·155	·0083	− 093	− 001	− 005
3–O	− ·216	·0084	− 011	001	− 005
4–O	− ·197	·0105	− 080	− 002	− 005
5–O	− ·201	·0054	− 107	− 004	− 003
1–A	− ·259	·0052	− 007	− 002	− 012
2–A	− ·353	·0015	+ 256	− 014	− 000
3–A	− ·291	·0028	+ 037	− 006	− 010
4–A	− ·192	·0007	+ 004	− 013	− 001
5–A	− ·298	·0047	+ 308	− 015	− 005
6–1	− ·197	·0073	− 092	008	− 004
6–2	− ·171	·0052	− 113	002	− 005
7–2	− ·140	− ·0070	+ 210	− 010	+ 001

Fig. 4 a.

Fig 4 b.

Fig. 4 c.

30—2
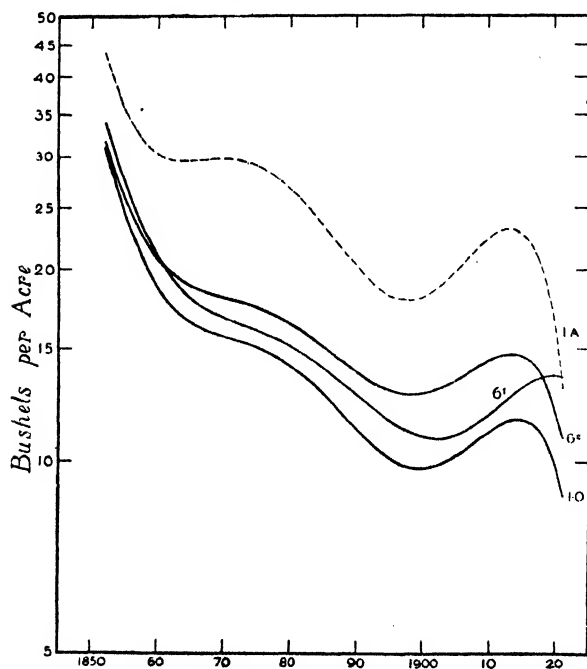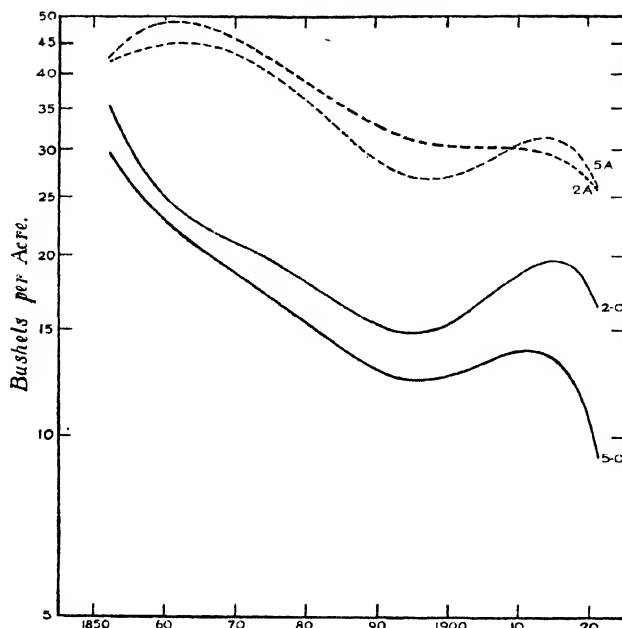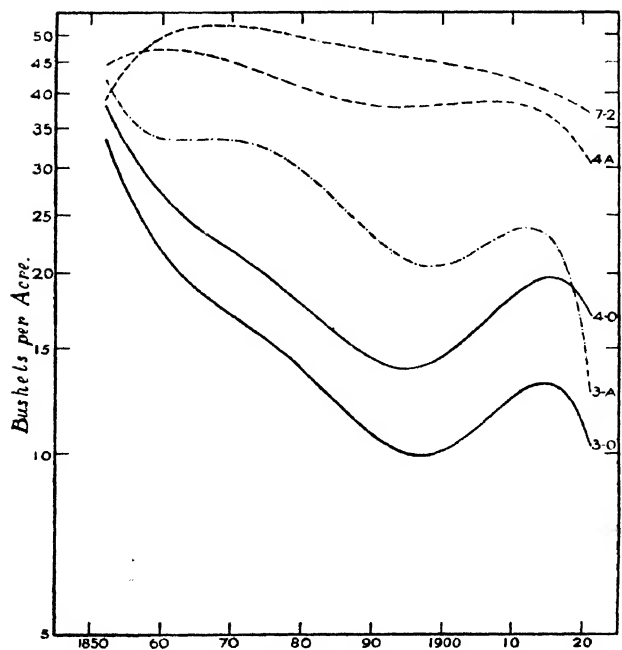
From the above table, and from Figs. 4*a*, 4*b* and 4*c* it will be seen that the curves fall into three main groups, viz.:

(1) Those in which the B and C terms only are significant, viz., "O" series, 6–1 and 6–2.

(2) Those in which the fifth term is significant, viz., 1–A and 3–A.

(3) Those in which the middle terms, D and E, are significant or probably so, viz., 2–A, 4–A, 5–A and 7–2.

The only curve about which there is any doubt in the classification is that of plot 7–2. Since only the first and second terms are decidedly significant, the curve undoubtedly belongs to the first class. The obvious difference between this curve and those belonging to the first group, as shown by Figs. 4*a*, 4*b* and 4*c* is due to the fact that though the parabolic term is significant in all cases, in plot 7–2 the term is negative, but in the other cases is positive. A comparison of the actual coefficients of the 7–2 polynomial with those of the other curves, indicates that possibly D and E are significant though the value of P for these terms would not in itself justify such a conclusion. Taking this into consideration, as well as the actual form of the curve, it has been decided to classify 7–2 under group 3.

After classifying the curves on the above principle, it was at once seen that the same groups are obtained by classifying the plots according to the manurial treatment as under:

(1) No nitrogenous manure, "O" series, 6–1 and 6–2.

(2) Nitrogenous manure but no phosphoric acid, 1–A and 3–A.

(3) Nitrogenous manure and phosphoric acid 2–A, 4–A, 5–A and 7–2.

This classification justifies the inclusion of 7–2 in group 3.

The relative unimportance of the slow changes may be seen from Fig. 1, and also from the combined significance of the third, fourth and fifth terms of the polynomial, as shown below:

Table XII.

Plot	Variance due to D, E and F terms only	P for D, E and F terms
1–O	1·90	·094
2–O	1·33	·527
3–O	0·83	·532
4–O	1·20	·792
5–O	0·97	·712
1–A	5·62	·058
2–A	3·02	·599
3–A	4·46	·170
4–A	2·25	·586
5–A	7·99	·030
6–1	1·77	·170
6–2	1·66	·341
7–2	3·11	·509

The great significance of the parabolic term may be judged from a comparison of the values of P for the three terms given above, and those given in Table IX. Unfortunately it is impossible to say how far the significance of this term is due to deterioration and how far to slow changes. From knowledge of the plots, and from inspection of the curves, one is led to think that in most cases the slow changes are less responsible for the significance of the parabolic term than is deterioration. This is confirmed by the relation of the slow changes to manurial treatment, especially in group 1, where the polynomial curves are seen to be fairly free from slow changes during the first 40 years and to represent the expected course of deterioration of plots starved for nitrogen.

The unimportance of the slow changes other than deterioration is in striking contrast to the Broadbalk results, where such changes were found to be very significant, and to follow the same course over the whole field. Moreover, in the case of the wheat plots, the slow changes tended to exaggerate the deterioration of those plots with the least diminution, but with the barley plots, the slow changes tend to obscure the full effect of the exhaustion of soil nutrients.

The nitrogen starved plots of group 1 show a fairly rapid deterioration during the first four decades, though less rapid at the end of the period than at the beginning. All plots within the group show an improvement in mean yield during a period from about 1895 to 1912.

Plots 1–A and 3–A, which form the second group, show a very rapid fall during the early years followed by a constant yield throughout the 'sixties. The next quarter of a century the mean yield of both plots was deteriorating at a fairly uniform rate, but during the later 'nineties a rise similar to that found in group 1 took place.

The chief characteristics of the third group of plots, and that which distinguishes the four curves comprising it from the other groups, is the increase in mean yield during the first years of the experiment. The increase is not uniform within the group, being decidedly more marked in the case of plots 2–A and 7–2. Moreover, the period of increase is longer in the case of the dunged plot, which does not reach its maximum until 1868, whereas the other three plots in the group begin to deteriorate between 1860 and 1863. The marked rise in the last years of last century, shown so strongly by groups 1 and 2 is only shown by one plot in group 3, viz. plot 5–A. Plot 7–2 shows no sign of even arrested deterioration, but the other two plots in the group—2–A and 4–A—show a constant mean yield during approximately the same period, 1895–1912.

These differences in the course of the slow changes on the various

plots is evidence that certain causes affecting the mean yield are even more local in their action than those of Broadbalk, being in some cases restricted to individual plots. It may be that the cause of the slow changes was different in different parts of the field; or it may be that in the case of some plots other influences were at work which tended to counteract the effect of the cause which in the case of other plots led to an increase in mean yield.

The records concerning the conditions of the plots and crops are exceedingly scarce during the early years, especially during the 'sixties. No information is available for these years until Mr Keenan's detailed notes of 1867, and similar records for 1868 and 1869. From this scanty data it is impossible to suggest any probable reason for the unexpected arrest of deterioration in plots 1–A and 3–A during the decade 1860–70.

On the Broadbalk wheat plots the course of the slow changes were general over the whole field, and the chief cause of the changes was found to be the prevalence or otherwise of weeds. With the barley plots, however, weeds do not seem to be the cause of the slow changes. The barley is not sown until March, or even April, so that each year the field has a winter fallow. The land is ploughed after harvest and again the following spring before the seed is sown, and hence the plots are not nearly so infested with weeds as are the Broadbalk plots.

From the yearly records of the condition of the plots, the land seems to have been fairly clean during the 'seventies, but during the 'eighties and early 'nineties the foulness of the various plots is constantly commented upon. Damage by wireworm and gout fly is also noted during the early 'nineties.

No records were made of the conditions of the plots in the later 'nineties from which we must assume that there was nothing worthy of note during the time. In 1900, however, we find "Notes upon the prevalence of the leguminous plant of *Medicago Lupulina*" which had spread rapidly over some of the plots, more particularly those starved for nitrogen. Apart from this detailed record, notes were made upon the general conditions of the plots, and the majority of them are spoken of as being "very weedy," or "foul with weeds." Six years later a special effort was made to remove the "couch grass and corn sow-thistle with which the land had become covered." The weeds continued to increase, however, and in 1912 the field was fallowed. It was during this period from 1895–1912, when the condition of the land seems to have been rather unsatisfactory that the improvement in the mean yield of most of the plots occurred. Had the rise been restricted to the plots starved

for nitrogen one would have been inclined to ascribe the increase in yield to an improvement of the nitrogen supply due to the action of the nitrogen-forming micro-organisms associated with the roots of leguminous plants. Such an explanation is impossible, however, in the face of a similar rise on plots 1–A, 3–A and 5–A, all of which are supplied with nitrogen in the form of ammonium salts, and on which no plant of *Medicago Lupulina* was found.

Earlier in this section attention was directed to the fact that plots 2–A, 4–A and 7–2 alone show no sign of a rise in mean yield: the two former showing a constant yield, and the latter continued deterioration. It is these same three plots which at one time or another are specially mentioned as suffering from wireworm attack during the period 1895–1912. Throughout these same years, whenever records were made, plot 7–2 was mentioned as being "weedy" or as having "much weedy rubbish growing through the laid corn." It may be therefore that there was a general influence operating over the whole field which tended to increase the mean yield during these years, but which was counteracted in the case of these three plots by the growth of weeds and wireworm attack.

SUMMARY.

1. Of the three sources of supply of nitrogen, rape cake gave the highest mean yields in the absence of superphosphate; while nitrate of soda gave results significantly better than those obtained from ammonium salts.

2. Superphosphate is of importance to the barley crop, giving greatly increased yield when applied.

3. Sulphate of potash seems to have an adverse influence upon the barley yield.

4. The deterioration of the barley plots is much heavier than on the wheat plots. The convention of ascribing the whole linear term to deterioration may not be as true for some plots as for others. Part of the diminution in mean yield may be due to slow changes other than deterioration.

5. The mean annual percentage diminution is least on those plots in receipt of superphosphate and emphasises the importance of phosphoric acid not only in increasing the mean yield, but in maintaining the fertility of the soil.

6. Barley is more variable than wheat and is more subject to the influence of meteorological conditions.

7. The slow changes other than deterioration are relatively unimportant and seem closely connected with the manurial treatment. The cause of the slow changes is obscure, but there were probably special influences operating on certain of the plots.

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MAIZE SILAGE. I.

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MAIZE is pre-eminently the best crop for silage in those parts of the world which favour its growth. Not only does it produce a very heavy crop of succulent and digestible forage, but by reason of its stout, erect growth, it can be cut and loaded very readily as well as rapidly fed and chaffed in the cutter. These factors render it cheap to grow and economical to handle per food unit.

In this country, however, attempts to produce silage from maize are by no means always successful. Frequently the resulting silage is excessively sour and unpalatable. The early investigations carried out in this connection by Annett and Russell (1) were by no means encouraging, since the average loss of dry matter in their experiments occasioned by fermentation and juice drainage amounted to as much as 36 per cent. of the original dry weight of crop ensiled.

On the other hand, there are on record instances of farmers in the southern counties who have been successful in producing good maize silage from crops which have attained a fair degree of maturity (*e.g.* cob formation) on the earlier soils of these warmer regions. Again, in many parts of Canada, where the growing period is comparatively short and the summer temperature not much higher than in this country, maize silage is successfully produced in large quantity.

The reason why the production of maize silage has not been generally successful in England would seem to be that the summer climate does not allow either a long enough growing period, or a sufficiently hot one, to bring the crop to the desirable state of maturity. It is now well recognised that if an ensiled crop be too immature and succulent, conditions arise in the silo which favour not only the production of an unpleasant and sour silage, but also the occurrence of large losses of dry matter as a consequence of excessive drainage of juice. If, with a view to lengthening the season of growth, the maize crop is drilled too early, the young plant is subject to the risk of destruction by night frosts. Even

though this danger be averted, the prevalence of low temperature causes growth to be very slow and, as a consequence, weeds become very troublesome. Moreover, early frosts in autumn frequently cut down the foliage before sufficient maturity is attained for the production of good silage.

Another and perhaps more important cause of failure is that the variety of maize grown in England (American Horse Tooth) though a bulky coarse growing variety, is one of the slowest to mature. It may possibly be a suitable variety for green soiling, though this is open to question, but there can be little doubt that it is a most unsuitable variety for silage in this country. Investigations are now in progress at Cambridge for testing the suitability of a number of varieties grown for silage in Canada and elsewhere, and these, though not complete at the time of writing, indicate the advantages attaching to certain varieties, *e.g.* Saltzer's North Dakota, Longfellow, Compton's Early and White Cap. All of these mature at least a month before American Horse Tooth and possess distinct possibilities in regard to yield of crop. If it is found that one of these definitely possesses all the necessary characteristics for producing a commercial crop in this country, then the possibility of extending the production of maize silage will be materially increased.

As a preliminary step in a series of trials designed to investigate the possibilities of maize as a silage crop in this country, it was decided to re-determine, on the lines of Annett and Russell's earlier work, the loss of dry matter sustained by green maize when converted into silage. It is obvious that if the loss is very much higher than that experienced during the ensilage of other crops such as oats and tares, as the results obtained by Annett and Russell would lead one to anticipate, then this would offer an appreciable discouragement to any efforts which might be made with a view to establishing maize as an ensilage crop in this country. On the other hand, reference to results obtained in the United States in this connection reveals figures which are more encouraging. At the Wisconsin Station, King [2] found that the losses of dry matter were reduced to a minimum by use of maize *of proper maturity* and by close packing into a silo having air-tight walls. He reported that in one typical silo where the dry matter losses were measured in eight layers of silage the average loss was 8 per cent. The loss in the surface layer was highest, 32·5 per cent., whereas that of the middle layer was least, namely 2·1 per cent. These losses were of about the same order as those observed in other cases where the silage was properly handled.

In order to institute a fair comparison between the present trial and previous work on the oat and tare [3], oat, tare and bean [4], and sun-

flower (5) crops, it was decided to make the maize silage in the wooden experimental silos (4 feet diameter and 6 feet in height) which had been employed in these earlier investigations. The filling of the silo took place on Oct. 31, 1922. The variety of maize grown for the experiment was American Horse Tooth. The variety was of necessity cut in an immature condition, very few stems even showing male inflorescences. It had been considerably damaged by frost in the preceding week and contained much withered leaf as a consequence. The conditions therefore of the trial were not too favourable to the production of a good quality of silage with minimal dry matter losses.

The crop was cut and carted to the silo on the day previous to filling. At the time of cutting, the material was free from rain and dew. It was allowed to remain in a heap overnight, but during this time developed no appreciable heat. The fodder was very wet to handle after chaffing and a moisture determination carried out on the analytical sample showed it to contain 16·52 per cent. of dry matter. The weight per acre of green crop was 14·6 tons (2·41 tons dry matter).

Two sample bags, each containing more than 40 lb. of the wet crop, were placed symmetrically in the silo, the one occupying the lower half and the other the upper half of the silo. The bags were surrounded on every hand by the compactly pressed fodder, and after the filling was completed in the manner described in a previous communication (4), the top of the silo was sealed with a 6-inch layer of soil.

The silo was opened on Feb. 1, 1923. The character of the silage in the upper bag was much better than had been anticipated, and it was quite free from all the unpleasant characteristics associated with the samples of sour silage usually obtained when immature green maize is ensiled. It was of a pale brownish green colour and combined a slightly acidic smell with a fresh odour suggestive of green maize itself. No smell of butyric acid could be detected. The character of the material was neither that of "sour" nor "sweet" silage. It contained 16·77 per cent. of dry matter. The silage from the lower bag resembled that of the first bag in every respect and was found to contain 16·65 per cent. of dry matter.

The analysis of the silage extracts and the silage dry matter was carried out by the methods described in detail in previous communications (3, 4). The dry matter sample was obtained by drying down a large weight of silage (about 1500 gm.) and finely grinding up the residue. The sample of green maize was obtained for analysis in a similar manner. The following tables summarise the results which were obtained.

Table I. Analysis of silage extracts (calculated on basis of 100 gm. dry matter).

	Bag 1 (lower half of silo)	Bag 2 (upper half of silo)
	%	%
Volatile organic acids*	1·38	1·33
Non-volatile organic acids†	3·19	2·86
Amino acids‡	3·23	2·94
Volatile bases‡	0·63	0·63

   * Calculated as acetic acid (control figure on green crop was negligible).

   † Calculated as lactic acid, after allowing for control figure on green crop. The non-volatile acidity for the green crop was appreciable, amounting to 8·2 c.c. normal acid per 100 gm. of moist green maize. Annett and Russell (1) noted that the juice from the maize fodder was usually slightly acid to litmus and showed that gallic acid was present, whereas lactic, malic, succinic and volatile organic acids were absent.

   ‡ Calculated as crude protein. The volatile base content of the green maize was negligibly small. The content of amino acids worked out at 1·06 per cent. (calculated as crude protein per 100 gm. of green maize dry matter).

Table II. Analysis of green maize and silage samples (calculated to dry matter basis).

	Green maize	Bag 1 (silage)	Bag 2 (silage)
	%	%	%
Crude protein	10·50	10·22	9·78
Ether extract*	2·06	3·93	3·57
N-free extractives	51·17	45·43	47·16
Crude fibre	25·77	30·15	29·23
Ash	10·50	10·27	10·26
True protein	7·53	6·42	6·28
"Amides"	2·97	3·80	3·50
Pepsin-HCl soluble protein	8·11	7·53	6·93
Protein digestion coefficient (*in vitro*)	77·2	73·7	70·9

   * Not taking into account volatile organic acids of silage.

Table III. Percentage gains or losses of constituents in Bag 1 (lower half of silo) and Bag 2 (upper half of silo).

	Bag 1			Bag 2		
	Green crop oz.	Silage oz.	% increase or loss	Green crop oz.	Silage oz.	% increase or loss
Moist material	715·00	602·00	− 15·8	673·00	586·00	− 12·9
Dry matter*	118·10	100·23	− 15·1	111·20	98·27	− 11·6
Organic matter* ...	105·70	90·08	− 14·8	99·52	88·32	− 11·3
Crude protein ...	12·40	10·10	− 18·6	11·68	9·48	− 18·8
Ether extract* ...	2·43	5·27	+117·0	2·29	4·75	+107·0
N-free extractives ...	60·43	44·91	− 25·7	56·90	45·74	− 20·0
Crude fibre	30·44	29·80	− 2·1	28·65	28·35	− 1·0
Ash	12·40	10·15	− 18·1	11·68	9·95	− 14·9
True protein	8·89	6·35	− 28·6	8·37	6·09	− 27·2
"Amides"	3·51	3·75	+ 6·8	3·31	3·39	+ 2·4
Pepsin-HCl soluble protein	9·58	7·44	− 22·3	9·02	6·72	− 25·5

   * Allowance made for volatile organic acids of silage as acetic acid. Amount of silage dry matter calculated as residue after drying at 100° C.: 98·85 oz. (Bag 1) and 96·98 oz. (Bag 2).

Comments on Analytical Results.

(1) *Juice drainage and dry matter losses.* The amount of juice which drained away from the bags during storage in the silo amounted to 95·1 oz. (Bag 1) and 74·0 oz. (Bag 2). This represented respectively roughly 16 per cent. and 13 per cent. of the juice of the green maize samples originally weighed into the bags. These unequal losses of juice explain the relatively high loss of dry matter from Bag 1 (15·1 per cent.) as compared with that from Bag 2 (11·6 per cent.), since the greater drainage entails higher losses of soluble constituents.

The use of small experimental silos is open to the criticism that the losses of dry matter measured in this way do not reflect accurately the losses which would occur if the same crop were ensiled in a commercial silo. Previous experience, however, has shown that such figures do indicate the order of the *average* losses sustained by the different layers of material throughout the big silo. In any case, the main advantage attaching to the use of the small silos is the feasibility of obtaining without difficulty comparative figures for the losses suffered by different crops during ensilage. It is therefore of interest to compare the losses which have been measured for the maize crop in the small silo with the results obtained for other crops in silos of similar dimensions.

	Oats and tares ("green fruity" silage)[3]	Oats, tares and beans ("green fruity" silage)[4]	Oats and tares ("acid brown" silage)[3]	Oats, tares and beans ("acid brown" silage)[4]	Sunflower silage[5]	Maize silage
	%	%	%	%	%	%
Top bag	13·4	8·6	9·1	—	—	11·6
Bottom bag	13·2	9·0	8·2	5·8	4·8	15·1

A survey of the above figures reveals the fact that the loss of dry matter associated with the production of maize silage is not appreciably different from that occurring during the making of "green fruity" oat and tare silage. In both cases, the large amount of drainage consequent on immaturity of the ensiled crop contributes materially to the dry matter losses. It is probable therefore that if a variety of maize can be found which is capable of being brought to the desirable stage of maturity before cutting for the silo, then the ensilage process should proceed with no bigger losses than those which characterise the production of "acid brown" oat and tare silage. Or, alternatively, if the drainage of juice can

be prevented altogether, then the losses of dry matter would be relatively small. In this connection, attention should be directed to the statement of Hall (6) to the effect that if a crop be ensiled in a concrete silo not provided with a drain, the expressed juice which otherwise would run off and be wasted is ultimately re-absorbed by the mass of silage. This points the way to the production of any type of silage with minimum losses, although it is not by any means certain that the retention of the juice in this manner would affect the palatability of the silage beneficially. Another proposal by the writers for preventing juice drainage consists of chaffing straw along with the green crop for filling into the silo, preliminary trials having shown that straw "admixes" satisfactorily with oats and tares in the silo. These and other questions concerning juice drainage are receiving attention at the present time.

The main conclusion arrived at as a result of the present experiments is that the production of maize silage is not necessarily accompanied by heavier losses than those occurring during the ensilage of oats and tares. It is largely a question of finding a variety of maize which will mature suitably under English climatic conditions.

(2) *Changes affecting individual constituents.* Reference to Table III shows that the material in both bags suffered considerable losses of crude protein, amounting in each case to about 19 per cent. of that originally present. The loss is not to be attributed directly to fermentation but to drainage, the juice carrying away in solution a large proportion of the soluble "amide" constituents of the silage. It is of interest to contrast the nature of the "amide" fraction of the green maize with that of the resulting silage.

	Green maize %	Bag 1 (silage) %	Bag 2 (silage) %
Total "amides" (crude protein—true protein)	2·97	3·80	3·50
Amino-acids*	1·06	3·23	2·94
Volatile bases*	Negligible	0·63	0·63

* From results of titration of extracts (see Table III).

The alteration of the chemical character of the "amide" fraction during ensilage is apparent from the above figures. In the green maize, roughly one-third of the "amides" were present as amino acids, whereas the amount of volatile bases was negligibly small. The "amides" of the silage are made up exclusively of amino acids and volatile bases (*i.e.* ammonium salts probably arising from hydrolysis of true amides). The big increase in the amount of amino acids arises from the hydrolysis of true protein. Table III shows that about 28 per cent. of the true protein underwent hydrolytic cleavage in the ensilage process, and the fact that the corresponding gain of "amides" amounted only to 6·8 per cent. in

Bag 1 and 2·4 per cent. in Bag 2 points clearly to a very substantial loss of the soluble amino acids in the drainage juice. It is to this circumstance also that the depression of protein digestibility (77 per cent. to 74 per cent. in Bag 1 and 71 per cent. in Bag 2) is to be attributed, since excessive drainage involves the loss of much pre-digested nitrogenous material.

The loss of carbohydrate as a result of fermentation amounted to 25·7 per cent. (Bag 1) and 20·0 per cent. (Bag 2) of the original amounts present in the bags. This result is very similar to those obtained in the production of "green fruity" oat and tare silage.

	Loss of carbohydrates %	
"Green fruity" oat and tare silage	(a) 19·5	(b) 19·7
"Green fruity" oat, tare and bean silage	(a) 20·1	(b) 24·9

The smaller losses of carbohydrates occurring during the making of "acid brown" silage from fairly mature oats and tares encourage the belief that the amount of carbohydrate undergoing breakdown might be reduced considerably if the maize were ensiled at a more mature stage.

In both bags, the fermentation of carbohydrate resulted in the ether extract being more than doubled in amount, owing to the formation of organic acids. As with all other samples of good silage so far investigated, the non-volatile lactic acid was present in excess of the volatile organic acids (see Table I).

The figures given in Table III do not show that the amount of crude fibre underwent any appreciable diminution during ensilage. It follows that the type of bacterial activity which results in the breakdown of the cellulose constituent was not much in evidence, although it has been shown in earlier work with oats and tares that the amount of fibre may be appreciably diminished by such action [3]. Annett and Russell [1] concluded that the less resistant cellulose appeared to be attacked by bacteria during the conversion of maize into silage, a result which was further confirmed by microscopic examination.

The appreciable loss of inorganic constituents (see Table III) during ensilage of the maize again directs attention to the drainage problem. That such losses of salts may be very significant from the nutritional point of view is shown by the recently published work of Godden [7] on the drainage from tower silos. If the valuable inorganic salts of the green crop are to be conserved, then drainage must be reduced to a minimum by one or the other of the methods previously discussed.

SUMMARY.

It has been shown that maize silage of good quality can be produced with losses of dry matter which are not necessarily much greater than those which characterise the production of oat and tare silage.

Excessive drainage of juice consequent on immaturity of crop contributes materially to the total loss of dry matter.

The main reason for the comparative failure of attempts in this country to produce maize silage successfully from the points of view of quality and economy lies probably in the general use of the late maturing variety, American Horse Tooth. Success will probably depend on growing a variety of maize which is able to reach a desirable stage of maturity under English conditions before being cut for the silo. Preliminary trials have indicated that the necessary qualities may be found in certain varieties like Saltzer's North Dakota, Longfellow, Compton's Early and White Cap, all of which mature at least a month before American Horse Tooth.

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A NOTE ON THE COLORIMETRIC ESTIMATION OF HUMIC MATTER IN MINERAL SOILS*.

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(With One Text-figure.)

ODÉN[1] has shown that colour intensity comparisons between alkali extracts of peat soils of varying origin can be made since, despite the possible difference in the nature of their humic matter, the absorption spectra are almost identical within the visible range. These absorption spectra are also comparable with that of Merck's Acidum Huminicum, which is a standard preparation. Odén developed his colorimetric method as a means of measuring the degree of humification of the organic matter of peat soils, that is to say, the proportion of the total organic matter which could be classed as humic matter. In this way the disadvantages of Grandeau's method, in which there is no discrimination between the coloured and the colourless parts of the soil organic matter, are obviated.

Odén's method depends upon digesting the soil at a high temperature with a strong solution of caustic soda. The actual colour intensity of the solution obtained is due, in part, to the readily soluble humus, and in part to an undetermined but in all probability large and fairly constant proportion of humin; the colour intensity of this solution may thus be taken as a fair measure of the amount of humic matter in the soil. The colorimetric method here described is an adaptation to mineral soils of the principle used by Odén. Following a series of experiments designed to determine the quantity of soil, concentration of reagent, and time of reaction which would give suitable extracts for colour comparisons, the following procedure was chosen as giving the closest duplicates when carried out on separate portions of the same soil.

Five grams of soil in a Gooch crucible were treated with 50 c.c. of 10 per cent. hydrochloric acid and then well washed. The filtrate was slightly coloured by the water-soluble material (fulvic acid of Odén), but this loss was not sufficient to affect the comparisons made later.

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* The term humic matter is used here as referring to the characteristic coloured organic matter of the soil which is made up of "humus" and "humin" (see Beckley, V. A., this *Journal*, 1921, **11**, 66).

[1] *Int. Mitt. Bodenkunde*, 1920, **9**, 391.

The soil was then transferred through a wide necked funnel to a 100 c.c. conical Jena flask previously calibrated with a mark giving the volume of the soil plus 100 c.c. About 60 c.c. of water were used for this operation; 20 c.c. of 50 per cent. caustic soda were then added by inserting the end of the pipette a little way below the surface of the water. By so doing a good meniscus was obtained free from bubbles. The flask was then filled to the mark with water, a few drops of alcohol being used to clear the meniscus if necessary. The flask was then immersed up to the neck in a water bath at 100° C. for 15 minutes, during which time the contents were constantly stirred. A portion of the hot solution was then filtered through a hardened filter paper (No. 50 Whatman) on a Buchner funnel. It is unnecessary and inadvisable to collect more than 20 c.c. of the extract as the pores of the filter paper rapidly become choked and the hot caustic soda loosens the fibres, thus rendering comparisons of the extracts difficult. Ten cubic centimetres of the cooled extract were diluted to 200 c.c. and used in the colorimeter. A soil of high humic content after this treatment shows the familiar light grey-brown colour of other soils poor in humic matter subjected to the same process, an observation which supports the view that most of the "humin" is changed into a soluble form.

The standard solution of Acidum Huminicum (Merck) was prepared from a sample which had been desiccated for 6 weeks and had ceased to lose weight. 0·3384 gram of this material was dissolved in a slight excess of caustic soda and made up to 100 c.c. This solution was thus $N/100$ according to Odén's determination of the equivalent of Acidum Huminicum. After intermittent shaking over a period of 48 hours no solid matter remained except a trace of a white sediment. The colour standard was a portion of this stock solution freshly diluted to a tenth of the original concentration.

Experience with many soil extracts showed that in strongly alkaline solution the colour intensity diminishes in the course of a few days through oxidation. All estimations were therefore made on the freshly prepared soil extract. This loss of colour was not noticed in the case of the less alkaline standard solution. In the colorimetric measurements the depth of the soil extract column was kept constant and thus the intensity of the colour of this extract was always expressed in terms of the standard. Where there was more than 25 per cent. difference between the two solutions compared, a further dilution either of the soil extract or the standard solution was made in order to make the comparisons more accurate. Each measurement was the average of ten readings. The

following data representing the comparison of a 50 mm. column of a soil extract with a similar one derived from another portion of the same soil, show the magnitude of the error encountered:

50·8, 50·8, 50·4, 50·3, 49·7, 50·3, 50·1, 50·0, 49·5, 49·4. *Average* 50·13.

The value of the method for comparative purposes was tested. The chief source of error lies in the choice of an arbitrary time of digestion. Two possibilities liable to vitiate results are:

(1) That in a given time for two different soils the colour intensity might have reached the constant phase for one of them but not for the other; in this case comparison would be inadmissible.

(2) That in a given time coloured inorganic substances (*e.g.* ferrates) might be brought into solution by the continued action of concentrated caustic soda solution at 100° C. Of the soils under consideration, the richest in humic matter was accordingly chosen and a time-colour intensity diagram constructed. The following data expressed in tenth percentages of the standard show the progress of extraction.

Time in minutes	0	5	10	15	20
Intensity	—	18·05	23·53	23·80	23·82
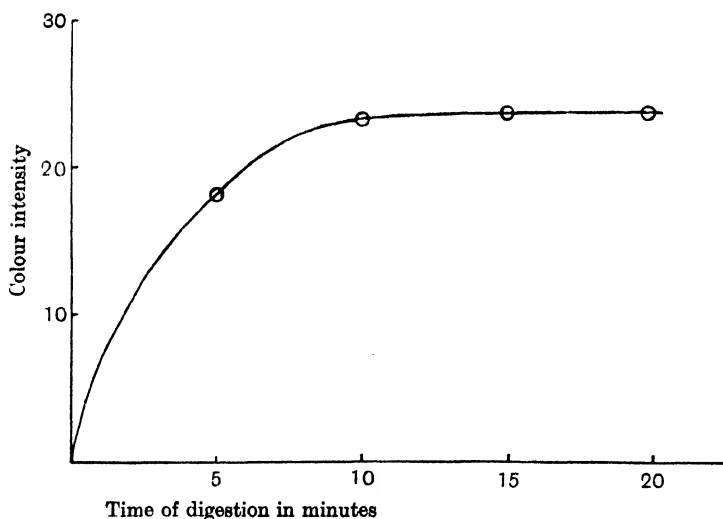
Fig. 1. Time-colour intensity curve for alkali extracts of humic matter.

At the end of 30 minutes' digestion the tones of the colours were no longer comparable. In the diagram a smooth curve has been drawn through these points. Since an extraction of 15 minutes seemed sufficient to allow the colour of the extract from a rich humic soil to attain con-

stancy and since no new point of inflexion appears on the curve, this time period was adopted. It is possible that with other soils slight modifications would have to be made in the quantities and concentrations of the reagents used, but the close approximation of duplicates, the regularity of the colour intensity curve, and the comparability of the tints themselves, indicate that in principle the method can be successfully applied to the estimation of humic matter in mineral soils.

From data of this type it is possible to calculate the amount of Acidum Huminicum which is colorimetrically equivalent to that extracted from the original weight of soil. This amount expressed as a percentage of the weight of organic matter in the same quantity of soil represents the degree of humification of the organic matter, that is, the Humification Number used by Odén.

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A PHYSICAL THEORY OF SOIL MOISTURE RELATIONS.

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(With Three Text-figures.)

(1) INTRODUCTORY.

THE following is a *résumé* of a physical theory of soil moisture relations developed by the author in 1920 [1]. On account of serious typographical and other errors in the original the essential argument is reproduced here together with some observations arising from subsequent work by other authorities.

A physical theory cannot hope *to predict* the behaviour of such a complicated medium as is, indisputably, the soil. At most it may be expected to determine the conditions under which empirical measurements will yield significant and comparable information. A model soil should not be expected to perform more than model mechanisms representing far "cleaner" and simpler processes, such, for instance, as take place within the atom. The treatment will be justified so long as the differential equations it enables us to construct throw light on the relationships which must exist between observable quantities, and thereby to realise to what extent the properties of moisture in soils are determined by specific characteristics of their constituents.

The model adopted pictures the soil as composed of discrete particles, each of which may be regarded as covered with a layer of colloidal material. Statistically, it is assumed that certain of the properties of such an aggregate can be represented by an assemblage of uniform spheres, packed in a regular manner; this does not imply that all the properties must be representable in the same manner. The validity of the assumption rests primarily on Green and Ampt's [2] experimental extension of the work of Slichter and King [3]. The mathematical basis of these investigations, it should be remembered, can only be tested practically by statistical methods involving volume relations such as the average area and length of a capillary pore, and the average diameter of the particles. In particular

no evidence is afforded of the average number of contacts between particles in a soil composed of grains of irregular sizes and shapes, a point which will be referred to later.

Experiment shows that there are two points at which a soil may be in equilibrium with an atmosphere saturated with water vapour in the earth's gravitational field. The first is at a moisture concentration determining the so-called Hygroscopic Coefficient; the second that attained by a saturated soil which is allowed to drain (the Moisture Holding Capacity). It might be assumed at first sight that since the soil under both these conditions is in equilibrium with the vapour phase, the same final equilibrium would be attained. This, in fact, has been assumed by Alway[4] who states: "Theoretically, actual equilibrium would not be obtained until the moisture content of the soil equalled that of the same soil in actual contact with water, but the time required for this is so great that this theoretical consideration does not affect the present discussion." The very large discrepancy between the points reached by approaching equilibrium from opposite directions, the air-dry and saturated states respectively, is thus attributed to a time factor. If, however, we assume that the two states differ from one another on account of the appearance of a new phase or phases, the existence of two real equilibrium points would become explicable. In the theory developed by the author it is suggested that the soil under the conditions defining the moisture holding capacity differs from those at the hygroscopic coefficient by the occurrence of two extra liquid phases, one the "free" water, and the other "vesicular" water contained in the colloid pores. The moisture content of the colloid net work will be the same under both conditions, and may be called the "gel" water; the additional water taken up by a saturated soil is to be referred entirely to the free and vesicular water. The latter is to be regarded as enclosed by the colloid and may differ in osmotic pressure from the free soil solution, equilibrium being maintained by the mechanical pressure of the semi-permeable colloidal reticulum.

(2) Thermodynamic Equilibrium in the Ideal Soil.

List of symbols used in mathematical parts of this paper.

a = average radius of soil particles.
A, B, C, etc. = universal constants.
A', B', etc. = specific constants.
c = number of contacts per particle.
n = number of particles per 100 gms. soil.
μ = moisture concentration per 100 gms. soil.

E =moisture concentration per 100 gms. soil at moisture equivalent.
H =   „   „   „   „   hygroscopic coefficient.
M =   „   „   „   „   moisture capacity.
S =   „   „   „   „   wilting point.
F =   „   „   „   „   maximum plasticity.
P =pressure (in general).
ϕ =   „   hydrostatic.
p =   „   vapour.
π =   „   osmotic.
ψ =   „   swelling, of a colloid gel.
ξ =colloid concentration per 100 gms. soil.
ζ =   „   „   per unit vol. colloid gel.
η =density of gas.
ϵ =   „   colloid gel.
ρ =   „   soil particles.
W =specific volume of water.
U =   „   „   „   in a solution.
X =   „   „   „   in a colloid gel.
V =   „   „   gas.
R =universal gas constant.
R' =gas constant for water vapour$=R/$mol. wt.
T =absolute temperature.
σ =vesicular coefficient.

In order to obtain a clearer insight into the conditions of equilibrium of these various states in which the water of the soil is supposed to exist, a thermodynamic cycle may be considered in which water is transferred through each phase isothermally. The method of the osmotic circuit developed by Callendar[5] enables us to write down the work terms involved in the circulation very simply. The phases are imagined separated by "vapour sieves"; circulation of the solvent in the isothermal system is produced by perfectly efficient and reversible machines M_1, M_2, M_3 etc., which are alone responsible for the external work involved. The work done in passing unit mass of solvent through such a machine, working between head and tail pressures P_1 and P_2 respectively, where the U's represent increase in volume of the phase produced by the addition of unit mass of water, is obviously

$$P_2 U_2 - P_1 U_1 + \int_{P_1}^{P_2} P dU = \int_{P_2}^{P_1} U dP \qquad \text{...(1),}$$

since

$$P_1 U_1 - P_2 U_2 = \int_{P_1}^{P_2} P dU + \int_{P_1}^{P_2} U dP.$$

We may therefore write down by inspection the integral on the right hand of (1) for the work done by each machine. It will only be possible to integrate these terms if we know the specific volumes U, V, W, X, etc.,

as functions of the pressures. For V the specific volume of the vapour, we may write $R'T/p$, on the assumption that the gas obeys Boyle's law. For the others in the absence of reliable knowledge we may use the mean values taken over the range of the integrals, and treat them as constants U', W', X'. For U' and W', the specific volumes of water and solution respectively, the error involved will not be very large, since the compressibility of water or a dilute solution is small; a more considerable error may however be involved in this treatment of X.
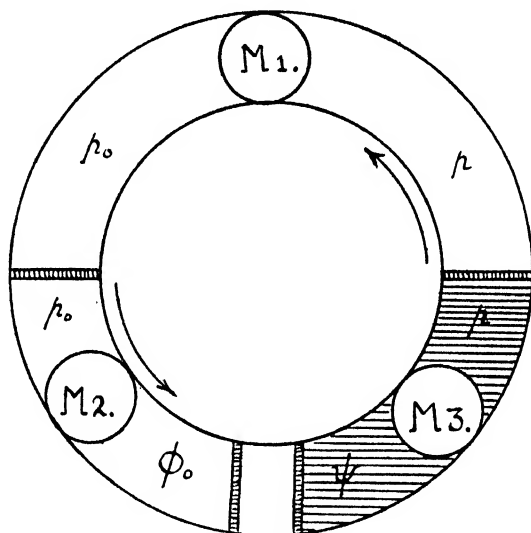
Fig. 1.

In the first case (Fig. 1) pure water, under hydrostatic pressure ϕ_0 is maintained in equilibrium with colloid, whose swelling pressure is ψ. For a circulation in the direction of the arrows the work terms will be

$$\int_{\phi_0}^{p_0} W'dP + \int_p^\psi X'dP + \int_{p_0}^p VdP = 0 \qquad \dots(2).$$

Integrating, and neglecting vapour pressures which will be small compared with the magnitude of ϕ and ψ, we obtain

$$W'(p_0 - \phi_0) + X'(\psi - p) + R'T \log_e \frac{p}{p_0} = 0,$$

or . $$X'\psi - W'\phi_0 = R'T \log_e \frac{p_0}{p} \qquad \dots(3).$$

At equilibrium, where $p_0 = p$, $X'\psi = W'\phi_0$.

By substituting a solution in place of water in Fig. 1, the only alteration in the integrals will be that W' is replaced by U', and ϕ_0 becomes ϕ_1. Thus

$$\int_{\phi_1}^{p_1} U'dp + \int_p^\psi X'dp + \int_{p_1}^p Vdp = 0,$$

whence
$$X'\psi - U'\phi_1 = R'T \log_e \frac{p_1}{p} \qquad \ldots(4).$$

Subtracting (4) from (3) we get

$$U'\phi_1 - W'\phi_0 = R'T \log_e \frac{p_0}{p_1} \qquad \ldots(5).$$

This means that the difference in the hydrostatic pressures, ignoring the correction due to U' and W', is equal to the true osmotic pressure π_0 of the soil solution.
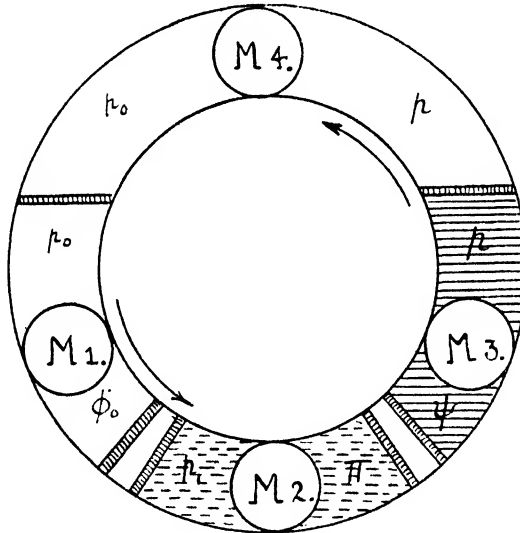
Fig. 2.

Fig. 2 will enable us to write down the conditions for the equilibrium of the four phases. As before the work terms will be

$$\int_{\phi_0}^{p_0} W'dP + \int_\pi^{p_1} U'dP + \int_p^\psi X'dP + \int_{p_0}^p VdP = 0,$$

whence $\quad W'(p_0 - \phi_0) + U'(p_1 - \pi) + X'(\psi - p) = R'T \log_e \frac{p_0}{p},$

or
$$X'\psi - W'\phi_0 - U'\pi = R'T \log_e \frac{p_0}{p} \qquad \ldots(6).$$

Subtracting equation (3),

$$X'\psi - U'\phi_1 - U'\pi = R'T \log_e \frac{p_1}{p}.$$

But at equilibrium the vapour pressure of the colloid must be equal to that of the solution, *i.e.* $p_1 = p$, therefore,

$$X'\psi - U'\phi_1 - U'\pi = 0; \quad \text{or} \quad \pi + \phi_1 = \frac{X'}{U'}\psi \qquad \text{...(7)}.$$

This means that if the hydrostatic pressure is zero, for equilibrium under any specified vapour pressure, ψ will only differ from $\phi_1 + \pi$ by the factor $\frac{X'}{U'}$. If ϕ is negative, as is the case when the moisture concentration of the soil falls below a certain value, as will be shown later, the osmotic pressure of the soil solution must increase. It is thus obvious that the colloid of the soil will exert an influence in determining the composition of the solution with which it will remain in contact when drainage takes place; absorption of salts by soil may thus be referred to the necessity of maintaining equilibrium according to equation (7) without any assumptions as to the "semi-permeability" of medium.

In order to investigate the application of these relations it will be necessary to devise indirect methods for the measurement of ψ, π and ϕ as functions of the moisture concentration in the soil. In the original paper, preliminary experiments in attempts to measure these factors were described; reference will be made here only to those experiments which have a bearing on the theoretical discussion. We may now examine how far the equations developed above may be expected to throw light on the quantitative moisture relations of soils; those which depend on the existence of a free liquid phase in the soil will be first investigated.

(3) The Free Liquid Phase.

For simplicity we will assume that the soil is composed of spherical crystalline insoluble particles, of uniform diameter and regular packing. Assuming the particles are wetted, the liquid will distribute itself in such a way as to reduce the liquid surface to a minimum. This will involve the collection of liquid at the points of contact of the particles. As a first approximation the surfaces of the menisci so formed may be supposed to be those of tangent spheres. The volume of the drop formed by the revolution of the triangle bounded by the two arcs, of radius

equal to that of the particles, and the arc of a tangent circle may be shown to be given by the expression

$$v = \frac{8\pi a^3 \sin^4 \theta}{\cos^2 2\theta}\left[1 - \left(\frac{\pi}{2} - 2\theta\right)\tan 2\theta\right] \qquad \ldots(8),$$

where 2θ is the angle subtended at the centre of the spherical particle by the radii from the point of contact and the tangent meniscus.

If we now make the assumption that the average number of contacts between particles in 100 gms. of soil is also related statistically with the average radius cubed, we can express the moisture content of a soil in terms of θ only. If the particles were true spheres of uniform radius the number present in 100 gms. of soil would be $\frac{100}{\frac{4}{3}\pi a^3 \rho}$, where ρ is the average density of the solid particles. The number of contacts per particle would also depend on the closeness of packing.

We are therefore justified in assuming that

$$\mu = nV = Cf(\theta) \qquad \ldots(9),$$

where C is a constant which depends only on the packing and possibly the shape of soil particles and not on their size as determined by statistical methods.

The hydrostatic pressure due to the surface energy of the drop may now be calculated from the well-known equation

$$\phi = -2\gamma\left(\frac{1}{r_1} - \frac{1}{r_2}\right)$$

by substituting for r_1 and r_2 in terms of the average radius and θ. The expression so obtained is

$$\phi = \frac{2\gamma \cos 2\theta}{a}\cdot\frac{\sin 2\theta + 2\cos 2\theta - 2}{(1 - \cos 2\theta)(\sin 2\theta + \cos 2\theta - 1)} \qquad \ldots(10).$$

When $r_1 = r_2$ ϕ vanishes. This is the case when $\theta = 26$ deg. 33 mins. nearly. The moisture content corresponding with this value of ϕ will then be a constant for all soils independent of the average diameter provided C determined by the packing is the same. If we put

$$C = \frac{8\pi a^3}{\frac{4}{3}\pi a^3 \rho}\times 100\cdot\frac{c}{2} = 1496,$$

i.e. the same value the constant would have if the spheres were all actually uniform of average density 2·4 and packed to minimum pore space when $c = 12$, the value of μ calculated for $\theta = 26$ deg. 33 mins. is 23·46. It was pointed out in the original memoir that this value is remarkably near that which appears as a constant term in the well

known relationship developed by Briggs and Shantz[6] between the moisture holding capacity (M) and the hygroscopic coefficient (H). This is: $M = 4 \cdot 2 H + 21$.

Keen[8] has objected that at moisture contents much below this, the values calculated would require the interference, or overlapping of the menisci of the individual drops. No reliance can therefore be placed on the approximation of the experimentally determined value with that calculated on the basis of uniform spherical particles.

The fact that this value is independent of the radius is, however, very significant. It is not to be expected that the absolute value of a constant depending on so many factors, which, in the model, are so enormously simplified, could be calculated otherwise than by an excessively complicated statistical investigation of the influence of grading of particles of irregular shapes and sizes on the number of contacts. The value must inevitably rest on empiricism for its magnitude, but the theoretical treatment justifies our regarding it as a true constant.

For any value of θ other than 26·3 the hydrostatic pressure will vary inversely as the average radius. If, however, we assume that other soil "constants" are determined by definite absolute values of the hydrostatic pressure of the free liquid, it is easy to see that the ratios of any two constants (in so far as they depend on the free liquid alone) in various soils will be the same. Thus since

$$\frac{\phi_1{}'}{\phi_2{}'} = \frac{\phi_1{}''}{\phi_2{}''}, \quad \frac{\mu_1{}'}{\mu_2{}'} = \frac{\mu_1{}''}{\mu_2{}''}.$$

The ratios of the moisture contents and the corresponding hydrostatic pressures for the two arbitrarily fixed points depend only on the value of ϕ. Empirically it is found that the wilting point (S) is related to the moisture equivalent (E) by the following equation

$$S = E/1 \cdot 84.$$

It is to be expected that with large variations in the colloid content and the specific nature of the colloid material, this relation, which only holds theoretically for the free liquid, may tend to vary; this point will be considered later. The fact that the mass of experimental evidence obeys this relation gives additional support to the correctness of the theoretical reasoning advanced.

(4) DISTRIBUTION OF WATER IN A VERTICAL COLUMN.

Here a simple relation may be deduced by equating the vapour pressure of the water in the soil to the equilibrium vapour pressure of water in the atmosphere, which will be determined by the height above the saturation level.

For the variation of the vapour pressure with height above a given level where $p = p_0$, we have

$$p = p_0 - g \int_{p_0}^{p} \eta \, dh \qquad \ldots(11),$$

where η is the density of the vapour and may be replaced by $p/R'T$; whence, on integration, we obtain

$$\log_e \frac{p_0}{p} = -\frac{gh}{R'T}.$$

For the relation between the hydrostatic pressure and the vapour pressure of pure water we have

$$\phi W' = R'T \log_e \frac{p_0}{p},$$

whence, equating to (11) $\qquad -\phi W' = gh \qquad \ldots(12).$

If we express ϕ in atmospheres and h in feet, the equation reduces to $-\phi W = kh$, where $k = 33\cdot8$.

No explicit relationship has been deduced connecting ϕ and μ. Corresponding values are tabulated below calculated on the assumption that $\psi = 71$ dynes per cm., and that C in (10) is approximately the same as that calculated on the basis of uniform spheres. The value of a is assumed to be 1 cm.; the value of ϕ for any other value of the average radius can be calculated by dividing the value of ϕ given in the table by the average radius in centimetres.

Table I.

θ	μ	ϕ (atm.)	h (feet)
5·0	0·07	890×10^{-5}	0·31
7·0	0·24	427	0·85
8·0	0·38	316	0·107
10·0	0·87	186	0·063
12·0	1·64	117	0·037
15·0	3·55	62	0·021
17·5	4·97	43	0·015
20·0	9·37	21	0·007
21·0	10·67	17	0·006
22·0	12·83	13	0·004
23·0	14·84	9	0·003
24·0	17·05	6	0·002
25·0	19·41	4	0·001
26·3	23·47	0	0·000

In Table II is given a comparison of the probable capillary rise in soils characterised by average size of particles shown in Column 1. Keen[7] has shown that Slichter's [3] conception of the triangular pore spaces in an ideal soil may be used on the capillary tube theory to calculate the height at which the soil would be saturated above that at which water would stand in a well. These values are shown in column 2. In column 3 is given the height at which the moisture content of the soil would be 5 per cent. calculated according to equation (12).

Table II.

Average diameter in mm.	Height above spring level at which soil will be saturated (Keen)	Height at which soil will be 5 %
1·0	0·3 feet	0·075
0·1	3·2	0·75
0·01	31·8	7·5
0·001	317·5	75·0
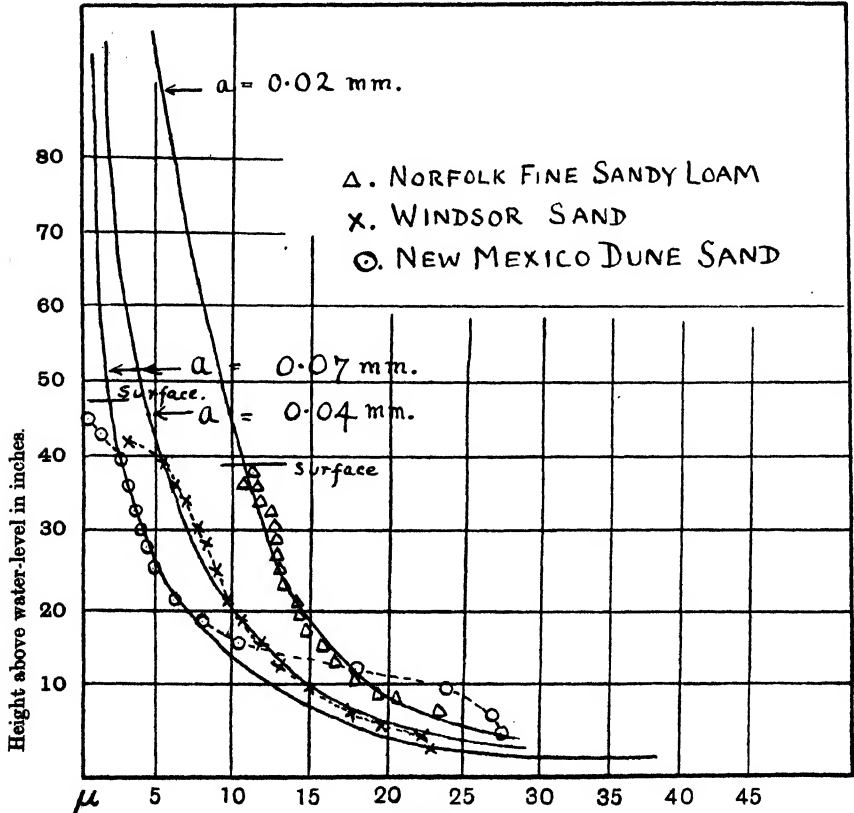
Fig. 3.

The figures in column 3 are more in accord with experience. Unfortunately there are at present no reliable figures by which the expression giving the variation of moisture with height above spring level might be tested. Buckingham's (9) figures are accompanied by no determinations of the average diameter or the mechanical analysis. Fig. 3 shows the theoretical curves fitted on that obtained experimentally with assumed values for a. Except with soils of the coarsest texture it will be seen that the fit is reasonably good and that the values of a assumed are of the right order of magnitude. The presence of a large amount of colloidal matter in the soil will cause little variation in the values of μ over the small range investigated and may be considered to affect the result by an additive constant only.

Another point which arises in connection with the free water of the soil is concerned with the theory of drain gauges and lysimeters, and does not appear to have received attention before. Since the hydrostatic pressure is negative for all values of μ below about 23, water can only be removed by the action of gravity when the pressure due to the weight of the drop is greater than that due to film curvature. A lysimeter will therefore give no evidence whatever of the drainage which may be taking place in an undisturbed column of soil due to a negative moisture gradient. This is supported by the fact that no drainage whatever took place from a drain gauge constructed in a dry soil at Lyallpur, until a quantity of water had been added, approximately sufficient to raise the whole of the isolated block of soil above its saturation point.

(5) The Soil Solution.

Equation (6) developed above shows that the osmotic pressure of the soil measured *in situ* will give only the apparent value. This fact does not appear to have been clearly recognised. Both the freezing point method of Bouyocos (10), the seed method of Schull (11), and the vapour method perfected by Thomas since the publication of the original of this paper (12), will give apparent values of the osmotic pressure which will depend both on the concentration of the colloid, and the texture of the soil as well as the concentration of the soil solution. While such measurements are of direct application for comparison with the agricultural properties of the soil in their static aspect, it is only by separating these several variables that we can hope to construct a theory of the part played by these forces in the dynamics of soil moisture. Thus the rate of movement of the free soil solution may reasonably be expected to be much greater than that of water in the colloid phase. If, however,

equilibrium between the colloid and the free solution with which it is in immediate contact, is maintained fairly rapidly, we may expect the colloid to play a determining part in the movement of the soil solution. This may be seen by considering the exchanges which may take place across the boundary of two soil strata, one of which for simplicity may be considered to contain no colloid. If we suppose the sand drier than the clay $(-\phi' > -\phi'')$, on bringing them into contact movements will take place tending to the equilibrium state at which the following equations will hold approximately,

$$\pi' - \phi' = \pi'' - \phi'',$$

$$\psi' = \pi' - \phi'.$$

This change, under actual conditions, may not, and probably will not, take place reversibly. The clay layer may in itself act as a partially permeable membrane. Thus, since we are justified in assuming that the movement of free liquid will take place by far the more readily, it is possible that pure water, or a solution containing the more mobile of the dissolved molecules of the soil solution, may move across the boundary to bring the hydrostatic pressure into equilibrium. The increased negative hydrostatic and osmotic pressures in the clay layer will then slowly extract water from the colloid, and this process will be accompanied by a slow diffusion of salt until osmotic equilibrium is reached.

Evidence that a process of this nature actually takes place was obtained in some preliminary work described in the original paper. Two quantities of a soil (clay 9·8 per cent., moisture equivalent 23·0, hygroscopic coefficient 2·43) were made up to water contents of 7·5 and 5 per cent. respectively. Corresponding with these, calcium ferrocyanide was added in another portion so that in the soils containing 7·5 per cent. moisture, the soil solution contained 20 per cent. of salt, while for the soils with 5 per cent. moisture the salt concentration was 15 per cent. The soils so treated were then pressed into flat slabs, and made up in pairs separated by parchment paper, in such a way that the initial moisture concentration was the same in both layers, but all the salt was in one. The pairs of slabs were then wrapped in tin foil and kept at constant temperature in an air-tight jar. The movement of water was estimated from time to time by cutting off a portion from the composite slab and analysing each layer. The following table shows the results.

Further experiments in which a copper ferrocyanide membrane was interposed between the layers enabled reliable determinations of the distribution of water at equilibrium to be made. The results cannot at

present be interpreted in the absence of reliable vapour pressure data for the same soil and soil solutions; the problem is further complicated by the fact that as the membrane will not be perfectly impermeable to all species of dissolved molecules, a complicated Donnan "membrane equilibrium" (13) will result, which with poly-valent ions such as are present with calcium ferrocyanide, will defy solution. A similar experiment with a non-electrolyte might give more satisfactory results.

Table III.

| | $\dfrac{\mu \text{ Salt layer}}{\mu \text{ Water layer}}$ | | |
Time	7·5 % water 20 % solution	5 % water 15 % solution	Remarks
0	1·0	1·0	—
4th day	1·20	1·10	No salt moved
6th day	1·40	1·30	„ „
8th day	1·30	1·20	Salt moved

The practical importance of a knowledge of osmotic action in soils will be realised by those acquainted with the problems of irrigation farming, under arid climatic conditions. While, on the one hand, economy of water is a first consideration, experience shows that a too parsimonious regime tends to render the soil saline. The soil proper is always distinguished from the subsoil by an increased humus content which appears to diminish linearly with the depth, and to which the moisture content at equilibrium is proportional. If we now suppose that the solution leaving the soil proper is in virtual osmotic equilibrium with the colloid, the *composition* of the drainage water should be related with the colloid content. Thus at the boundary of the soil proper we may suppose that the water table imposes an equilibrium value of the hydrostatic pressure ϕ_0, the true osmotic pressure π_0 of the soil solution being regarded as approximately constant. (This follows from the fact that U' and W' in equation (5) are practically identical since the solution is dilute.) For drainage to commence $\phi' > \phi_0$ and since, except with an excessively heavy irrigation, ϕ will be negative,

$$- \phi > \psi_0 - \pi_0.$$

This means if ψ_0 can only attain equilibrium slowly, that π_0 must increase. The colloid will therefore act as a regulator of the composition of the drainage water. In an entirely crystalline soil the drainage water will have the same composition as that applied in irrigation. With a heavy soil the drainage water will be less concentrated than that left in the soil proper. It would therefore appear from this cursory examination

that in sandy soils, frequent and light irrigations would more tend to economy without endangering salt accumulation.

With a heavy soil, on the other hand, in order to remove accumulated salts heavy irrigations must be given. Drainage in a comparatively dry colloidal soil leaves the salts behind.

(6) The Colloid Phase.

Experimentally we find that the equilibrium moisture content of a soil in contact with a liquid is not the same as that attained when contact is through the vapour phase. Theoretically this would indicate that there are phase differences between the two points. If we suppose that the hygroscopic coefficient is defined by the equilibrium of vapour and gel, regarding the system as containing the two components, water and colloid only, by fixing the temperature and vapour-pressure, the system will be defined.

If there is a free liquid phase we must now consider the system one of (at least) three components, since the concentration of the water in the gel phase, and the concentration of salts or dispersed colloids in any solution, will be separately variable. Fixing the temperature will now fix also the vapour pressure since we assume the presence of a free liquid phase. Equilibrium of (at least) four phases will thus be required for determining an invariant point, *i.e.* vapour, gel, liquid, and one other. In the original memoir it was suggested that the fourth unknown phase is a second liquid phase enclosed in the colloid mass. This phase, which was termed the "vesicular" phase, will probably have an osmotic pressure different from that of the free liquid, under a different hydrostatic pressure exerted by the walls of the colloid retaining structure. The empirical relationships of Briggs and Shantz support this view. In their equation

$$M = 21 + 4 \cdot 2 \, (H),$$

it was suggested that the variation in moisture content, due to the vesicular phase, is represented by the factor $4 \cdot 2$ which was called the vesicular coefficient. It was recognised that the value of this factor would be likely to vary with the specific nature of the colloid. It is also to be noted that the vesicular coefficient will be a variable, related with the hydrostatic and vapour pressures in such a way that the variation of ψ will be determined by functions of both the vesicular and gel concentrations.

Experimental evidence was obtained by measuring the positive absorption of water by soil suspended in sugar solutions. The curve extrapolated to zero concentration, indicated an absorption of $11 \cdot 5$ gms.

water per 100 gms. soil. The hygroscopic coefficient of the soil was 2·43. The ratio 11·5/2·43 = 4·73 is near the average value 4·2 of the Briggs-Shantz formula. The method is open to many objections, and the accuracy is small since the result depends on the determination of small differences of concentration. Subsequent work, not yet published, also shows that the values obtained are not independent of the proportions in which the soil and solution are mixed. This would indicate a positive absorption of sugar, which would introduce serious error in dilute solutions with large amounts of soil. It is considered, however, that the evidence of variation of the vesicular coefficient with the osmotic pressure of the solution in equilibrium is trustworthy, although the method does not commend itself for the absolute determination of this magnitude.

Hardy [14] has considered this explanation of the Briggs-Shantz empirical relationship from the point of view of the moisture content of soils at the point of maximum plasticity. This soil "constant," determined by the onset of stickiness on moistening an air dry soil, is taken to represent the point at which the free liquid films which are responsible for the internal tenacity of the structure are only just beginning to be formed. He assumes that when the colloid material of the soil is fully saturated with water, external adhesion then, and only then, becomes manifest. Hence the moisture content of a soil at the onset of external adhesion (the point of "stickiness"), is taken as a measure of the total imbibition capacity of the colloid content.

On this hypothesis Hardy identifies the vesicular coefficient with the ratio

$$\frac{\text{Moisture content at maximum plasticity}}{\text{Hygroscopic coefficient}} = \frac{F}{H} = \sigma.$$

In various soils of different geological type experimentally determined values were found to range between 4·68 and 2·37, the lowest value being that of a red laterite soil. The ratio F/H appears to diminish with increasing H in all cases except that of a very abnormal soil which contains 58 per cent. humus and has a moisture holding capacity of 254·9.

In order to obtain some idea of the extent to which Hardy's "constant" F may be regarded as specific we may assume that the point is defined by a critical value of the hydrostatic pressure. Hardy assumes that there is no free water at this point, but it is difficult to see how the soil can flow without a free liquid phase. In what follows specific constants, *i.e.* those which will depend on individual properties of soils, are indicated by a superscript dash.

For the moisture content of the colloid (gel + vesicular) an empirical

equation of the form $\psi = k\zeta^n$ was found by Ostwald [15] to represent the variation of swelling pressure with concentration of several colloidal materials, and enables us to deduce an expression of the form

$$\mu_o = \xi \left(\frac{k\epsilon}{\psi^{1/n}} - 1 \right) \qquad \text{...(13),}$$

where ξ is the colloid concentration per 100 gms. soil, and ϵ is the density which will be a function of ψ. Since

$$\frac{X'}{U'} \psi = \pi + \phi,$$

we may substitute the critical value of ϕ, which determines the value of F, on the assumption that π is small and varies little from soil to soil. The expression on the right of (13) will thus reduce itself to a specific constant which we may write $A'\xi$. For the free liquid we may use an empirical equation which agrees reasonably well with the figures calculated in Table I. This is of the form

$$\frac{\phi}{a} = B \cot \kappa\mu \qquad \text{...(14).}$$

The free moisture may thus be expressed in terms of another specific constant, B', as follows

$$\mu_f = f \frac{\phi}{a} = B_a'.$$

This, of course, involves the assumption, which is not strictly true, that the average radius of particles is independent of the moisture content. The hygroscopic coefficient will be a function of ξ alone, so we may write the expression for the moisture content at the point F,

$$\mu_F = \mu_f + \mu_o = B_a' + A'\xi = B_a' + fA''H.$$

$\sigma = \dfrac{\partial \mu_o}{\partial H}$ is thus a variable, independent of a, and determined by the properties of the colloid alone. F, however, will vary with the average diameter as well as the vesicular coefficient. This is supported by an examination of the figures published by Hardy. His soils of Type 1 a fall within narrow limits on the curve

$$F = 3\cdot43\,H + 9\cdot64.$$

Here the vesicular coefficient is $3\cdot43$ as compared with a value of about $5\cdot4$ at saturation. Table IV gives the values of σ and μ_f at the points F and M, together with the mean probable errors (q).

Table IV.

Soil type	σ_F	μ_{fF}	q	σ_0	μ_{f0}	q
1	3·43	9·64	±0·26	5·4	20·5	±0·62
2	3·01	9·10	0·74	5·28	19·8	0·8
3	2·70	10·10	1·10	5·46	20·9	1·2
4	2·83	9·80	0·53	3·61	21·5	0·4

The fact that in all four types of soil the free water at the point F is round about 10 per cent. would indicate that the average diameter does not vary very much. There appears to be no definite relationship between σ_F and σ_0. This would only be expected if the colloidal material in each soil type had identical properties.

If the hypothesis on which this discussion is based is correct, namely, that at the point of maximum plasticity the free water of the soil attains a definite value, the determination of vapour pressures at this point for all soils should show identical values, or, more accurately, the ratio p/p_0 should be constant. This criterion should hold equally for any soil "constant." If this supposition is found to be the case we should be on certain ground in assuming that F has a real significance in the properties of soils, and the ease with which it is determined would have many advantages in their practical study. The number of measurements it will be necessary to make in order to find an expression of the nature of equation (13) which will connect ψ with μ_c can only be determined by further investigation. In this connection it may not be premature to suggest that for the physical characterisation of soils a knowledge of the value of a and a function giving the variation of σ with p or ϕ should provide most of the information necessary. For the determination of a, that of Green affords a suitably convenient method. If another point, determined by constant ϕ were available, this together with F and M would probably be found sufficiently to define the ψ/μ curves.

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THE COMPOSITION OF SOME SUDAN SOILS.

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THE object of the present communication is to record the mechanical and detailed chemical composition of some typical soils of the Sudan, which in many respects differ from any for which full descriptions are easily accessible in the literature.

1. GEOGRAPHICAL NOTE.

The Anglo-Egyptian Sudan has an estimated area of a million square miles lying roughly between latitudes 22° and 4° north and meridians 22° and 37° east.

It is bounded on the north by Egypt, on the east by the Red Sea, Eritrea and Abyssinia, on the south by Uganda and the Congo Free State and on the west by French Equatorial Africa. The White Nile, which rises in the Uganda lakes, is reinforced by numerous tributaries as it flows north to Khartoum where it meets the Blue Nile, which, rising in Lake Tana, flows north-westward from 'the Abyssinian Hills. From Khartoum, the main Nile flows north, receives the Atbara, which is its last tributary, and passes through Egypt to the Mediterranean Sea.

The Sudan is divided into fifteen provinces from thirteen of which soil samples have been examined during recent years. The range of climate over this area is very great, the rainfall varying from practically nothing in the north to 1200 mm. in the south.

2. SOIL TYPES.

Up to the present no attempt has been made at a systematic agricultural or soil survey of this large country, but sufficient information has now been obtained to enable a description to be given of the more important soil types, which are shown in the table below.

Table I.

Name*	Source	Colour	Calcium carbonate	Composition	Remarks
1. River alluvium	River deposits: banks and basins	Brown	Low	No stones and gravel. Highly siliceous coarse sand. Much clay and silt	Soluble salts low
2. Flood alluvium	Thick annual deposit from Abyssinian hills flood water	,,	,,	,,	,,
3. "Badob†" or cotton soil	Loess: Aeolian deposit from northern desert	,,	As black calcareous nodules and coarse sand	High in clay and low in silt	Soluble salts high
4. Khor soil	Alluvial deposit from khor water	Almost black	Small amounts in coarse sand and silt	Very high in clay: very little coarse material	Soluble salts low
5. Goz soil	Aeolian sand deposit from northern desert .	Reddish brown to brick red	None	No stones and gravel: much coarse and fine sand	Soluble salts extremely low
6. Blue clay soil	Loess: similar to (3)	Slate blue or grey	Very low in clay and silt fractions	High proportion of clay	Soluble salts low

* The names given here are those we have been in the habit of using locally. They are not necessarily those which will be finally adopted as type-names.

† Pronounced with both vowels long.

(a) *River alluvium.* These soils occur as strips parallel with the river and usually extend only a short distance—perhaps a mile—from the river bank. Considerable areas exist in the so-called "basins" which are depressions annually flooded by the river.

The soils consist of brown clays and silts: stones and gravel are always absent and the proportion of coarse sand is low.

These soils are cultivated in almost all provinces through which the Nile or one of the larger tributaries pass and basin cultivation is carried on in the provinces of Berber and Dongola. The old alluvium (or land which once formed the river bank) is called "karu" to distinguish it from areas which are still being flooded annually.

(b) *River flood alluvium.* In certain parts of the eastern Sudan, the rivers from the Abyssinian hills discharge annually a rapid and turbid flood on to the land. The deposit thus laid down, which of course varies greatly with the force of the current, is similar to that of river alluvium.

The important areas of Tokar (Red Sea Province) and Kassala (Kassala Province) are cultivated by means of the Baraka and Gash floods respectively.

(c) *"Badob," Loess or cotton soil,* are names given to a heavy brown clay soil of aeolian origin. It is characterised by a high proportion of clay and a low one of silt. In the most important areas examined, the

stones and gravel consist largely of black nodules composed almost entirely of calcium carbonate. This soil is often of considerable depth and it appears to have little tendency to drain even when resting on sand. It usually contains a high proportion of salts soluble in water. The proportion of clay is higher in the southern than the northern districts.

The most important area of badob is the "Gezira" lying between the Blue and the White Niles in the Blue Nile Province. Grain and other crops are grown under rain cultivation, but cotton depends almost entirely upon irrigation from the Blue Nile. A description of the Gezira with soil analyses was given some years ago by Beam [1].

(*d*) *Khor soils*[1]. This term is used to denote an alluvial deposit left by khors either as the dried up bed or as a deposit on the sides. In many properties it resembles a heavy alluvium but those examined were much darker in colour than either the Nile alluvium or the loess.

These soils are wide spread: those examined in the laboratory have been mainly from Kordofan.

(*e*) *Goz soils*. These are red sandy soils consisting mainly of "sand." Stones and gravel are absent and the clay varies from 5 to 50 per cent.: they vary in colour from reddish brown to brick red. They are cultivated under rain only.

The whole of the gum districts of Kordofan consist of this type of soil.

(*f*) *Blue clay soils*. The soil in the vicinity of the upper reaches of the White Nile and its tributaries often consist of a heavy clay soil of a slate blue colour. It always contains a high proportion of clay, sometimes as much as 75 per cent. Stones and gravel are absent and the soluble salts usually low.

3. METHODS OF EXAMINATION.

Mechanical analysis is carried out as described in a former publication [2]. Nitrogen, phosphorus and "soluble" potash have been estimated by the usual methods, the two latter being extracted by boiling 20 per cent. hydrochloric acid, but the difference of opinion which exists as to the interpretation of the results have prevented us from making the determinations in large numbers.

"Soluble salts" are determined approximately by measuring the specific conductance of an extract made with one part of soil and five of water and multiplying the result by 250 (see [3], p. 52) and the alkalinity by measuring electrometrically the hydrogen-ion concentration [4]. In

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[1] Khor—the temporary channel of a running stream during the rainy season.

many cases moisture equivalents have been determined and some general results are given in a recent paper (3).

The most important data in the present communication are the results of the full chemical analyses which have been made of six soils using the methods of the Geological Survey of the United States (5).

In examining the general features of the different soils, samples are usually taken each foot from one to four feet, but in some cases the sampling has been extended to 12 feet, to test particularly the vertical distribution of soil alkali. The full chemical analyses have been made on first foot samples.

4. SOILS EXAMINED.

The chemical analysis of a whole soil gives very little information as to its character: for example, a soil containing coarse quartz sand and fine fractions low in silica might have the same composition as one containing no coarse sand but composed of fine fractions high in silica. For this reason, the soils chosen for ultimate analysis have first been separated into the conventional fractions by the ordinary methods of mechanical analysis and each fraction analysed separately. Whilst this increases the labour of the investigation so greatly that only a few soils have been completely examined it is felt that it is the only method likely to be of any use in characterising a soil.

The soils which have been analysed are as follows:

Table II.

Type		Flood alluvium	Badob	Khor soil	Goz soil	Blue clay soil
Locality	...	Kassala	Gezira Blue Nile	Um Ruaba Kordofan	Abu Haraz Kordofan	Nasser Upper Nile
Colour	...	Light brown	Dark brown	Black	Red	Slate blue
Stones and gravel %		0·0	2·7	0·0	1·1	0·0
Coarse sand %		5·4	10·0	1·7	31·9	5·2
Fine sand %		15·7	19·2	15·2	34·8	13·4
Silt %		32·2	10·8	15·1	2·8	13·8
Clay %		46·7	57·2	68·1	29·5*	67·6
Soluble salts %		0·028	0·100	0·035	0·005	0·029
pH		8·3	9·4	8·1	8·5	8·5

* This sample was selected in order to obtain more clay than is usual with this class of soil: almost 10 % is a more common proportion.

5. GENERAL CHARACTERS OF THE SOILS.

None of the above types are common in temperate climates: they appear to be associated with a country consisting of large uniform plains with only the gentlest slopes. On the other hand, in the south and west

of the Sudan, soils are met with which show a much greater variety in accordance with the more varied topographical features: large stones, calcareous nodules, and gravels are more common and types more like those found in Europe are more frequently met with. As these areas are less important from the point of view of immediate agricultural development, very little work has been carried out on them up to the present.

All the above soils are alkaline, having pH values varying from 8 to 9·5, and all are low in organic matter which only occasionally exceeds 1 per cent. The nitrogen is low, usually less than 0·05 per cent. and of this only one-tenth is likely to be in the form of nitrates. Nitrification in arid soils has been the subject of numerous investigations and an account of experiments carried out in recent years near Khartoum has been published lately(6).

The phosphoric oxide extracted by 20 per cent. boiling hydrochloric acid varies between 0·1 and 0·2 in first foot samples.

6. Results of the Chemical Analysis.

The detailed results of the analysis are given in Table III below. From these figures together with those for the mechanical composition of the soil, it is, of course, possible to calculate the percentage composition of the whole soil, but such results are of so little value, that this has not been done.

Table III.

	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	MnO	CaO	MgO	Aq–Org	CO_2	Na_2O	K_2O	Total
						Gezira						
St. and g.	7·69	5·67	1·23	0·57	2·32	46·36	0·29	—	36·33	—	—	100·46
C. sand	10·38	6·49	1·32	0·50	1·18	44·86	0·62	—	34·65	—	—	100·00
F. sand	75·62	6·34	7·98	2·30	0·50	4·49	0·76	0·97	0·56	0·50	0·03	100·05
Silt	53·67	12·76	10·32	5·95	—	6·25	2·31	6·42	1·52	0·36	0·21	99·77
Clay	46·70	20·22	12·44	2·03	—	1·10	1·59	15·16	—	0·48	0·54	100·26
						Um Ruaba						
C. sand	85·25	2·48	1·30	0·28	0·14	6·11	0·16	1·12	4·06	0·71	0·13	101·74
F. sand	81·40	7·56	2·82	1·72	0·97	2·12	0·30	1·82	0·24	0·68	0·33	99·96
Silt	61·92	12·79	5·68	2·30	0·37	5·17	0·53	5·56	2·44	0·36	1·08	98·20
Clay	48·35	20·72	13·14	2·90	—	1·76	0·56	12·66	—	0·79	0·35	101·23
						Nasser						
C. sand	88·79	5·09	1·63	0·63	0·29	1·21	0·19	1·45	0·06	0·57	0·59	100·50
F. sand	73·57	9·53	4·95	2·40	1·32	3·57	0·60	2·61	0·44	0·98	0·67	100·64
Silt	57·39	14·95	6·87	5·17	—	4·66	0·76	8·73	1·41	--	0·07	100·01
Clay	48·77	17·27	15·48	3·12	—	1·35	0·80	12·97	—	0·17	0·38	100·31
						Abu Haraz						
C. sand	98·03	0·34	1·06	0·29	—	0·45	0·02	0·73	—	0·52	0·08	101·52
F. sand	94·47	1·39	1·09	1·72	0·02	0·63	0·33	0·77	—	0·44	0·56	101·42
Silt	63·54	11·14	9·87	3·94	—	2·23	0·60	8·73	—	0·53	0·15	100·73
Clay	42·08	25·10	14·38	2·78	—	0·80	0·64	13·12	—	0·51	1·28	100·71
						Kassala						
Silt	49·86	20·45	9·61	1·42	0·36	2·60	3·98	8·84	—	1·49	1·89	100·50
Clay	44·58	19·98	14·41	1·42	0·24	1·18	2·95	14·17	—	0·18	1·35	100·46

7. SUMMARY OF THE RESULTS OF THE CHEMICAL ANALYSES.

Table IV contains the salient features of these results.

"Other oxides" includes those of aluminium, iron, titanium, manganese, magnesium, sodium, potassium and calcium, the latter reduced by the quantity equivalent to the carbon dioxide found, this amount being assumed present as calcium carbonate.

Table IV.

	Kassala	Gezira Blue Nile	Um Ruaba Kordofan	Abu Haraz Kordofan	Nasser Upper Nile
Stones and gravel	None	Calcium carb.	None	None	None
Coarse sand	Not analysed	80 % calcium carb.	85 % silica	98 % silica	89 % silica
Silica fine sand	Not analysed	76	81	94	74
Other oxides	—	22	16	6	23
Silica silt	50	54	62	64	57
Other oxides	41	37	26	28	31
Silica clay	45	47	48	42	49
Other oxides	42	38	40	45	38

Dealing with the fractions in order, the results show that

(a) The Gezira badob soil alone contains particles above 2 mm. in diameter and these consist mainly of calcium carbonate.

(b) The coarse sand fraction consists of silica, except in the Gezira soil. In this it consists of about 80 per cent. of calcium carbonate, the remainder consisting not of free silica but of silicates somewhat low in silica.

(c) The fine sand fraction consists mainly of silica with about 15 per cent. of basic oxides, except in the case of the Abu Haraz red soil where it is 98 per cent. silica.

(d) The silt fractions of the Gezira and Nasser soils are very similar, containing about 55 per cent. silica and 30 per cent. basic oxides: the two quite different soils from Kordofan (Um Ruaba and Abu Haraz) have very similar silt fractions, containing about 63 per cent. silica and 23 per cent. basic oxides.

(e) The clay fractions from the Gezira, Um Ruaba and Nasser blue clay are similar, with about 48 per cent. silica and 38 per cent. basic oxides. The clay from Kordofan red soil is quite different having 42 per cent. silica and 45 per cent. basic oxides, whilst that from Nassala occupies an intermediate position in this respect.

8. Composition of the Clay Fractions.

Clay plays so important a part in most of these soils that a few additional remarks may be made on the differences as shown in the complete table of analyses. The relation between the physical properties and chemical composition of clay is a very complex subject: a considerable amount of information has been collected on it in these laboratories, which it is hoped to publish in due course, from which it appears that a very important factor in determining the plasticity of a clay is its composition as shown by the molecular ratio of silica to alumina.

For the soils now described the figures are as follows, it being understood that the remarks on the subject of plasticity are only derived from a general impression as to their behaviour in the field.

Table V.

Clay from	Silica-alumina ratio	Plasticity
Kassala	3·77	Fairly plastic
Gezira	3·90	Plastic
Um Ruaba	3·95	,,
Abu Haraz	2·85	Only slightly plastic
Nasser	4·79	Very plastic

The above figures are suggestive and the subject is being more fully investigated in the laboratory: it may be added that the almost non-plastic mineral kaolinite has a silica-alumina ratio of 2·0.

Summary.

A description is given of five typical soils of the Sudan covering a wide area. Of these, four are heavy clays and the fifth a sandy soil. All are alkaline and with one exception low in calcium carbonate. The results of the full chemical analysis of the conventional fractions are given and it is suggested that an important characteristic of a clay soil is the chemical composition of the clay fractions.

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DIGESTION TRIALS WITH SWINE.

I. DESCRIPTION OF HARNESS AND METABOLISM CRATE.
II. DIGESTIBILITY OF BARLEY MEAL.

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(With Two Text-figures.)

INTRODUCTION.

THE carrying out of digestion experiments with swine introduces difficulties of technique which are not encountered in similar work with sheep. The pig is apt to be restive under restraint and does not always take kindly to the wearing of harness. The designing of a suitable harness is not easy on account of the shape of the pig, and when confined in a metabolism cage, the animal frequently spends much of its time rubbing vigorously against the sides, thereby considerably disarranging the harness. Moreover, since the pig increases in size so rapidly, it is necessary that the fit of the harness be capable of adjustment within wide limits. A further difficulty is connected with the voracious appetite and destructiveness of the pig, so that if the experimental ration does not satisfy the hunger of the animal, the latter will often gnaw the wooden parts of the crate and will even devour its own faeces, if the degree of freedom of movement permits it to turn round. The quantitative feeding of a pig is a matter of difficulty, owing to the animal's eagerness and its habit of stepping into the food trough. Swine do not display such hardiness under confinement as do sheep, and it is therefore necessary to maintain an equable temperature in the metabolism room throughout the experiments.

In his classical researches on the metabolism of swine, Meissl (1) did not attempt the use of harness, but constructed a cage in which the animal could lie down comfortably and could move backward or forward

to the distance of about 18 inches. It could not, however, turn round. The floor of the cage consisted of glass plates, suitably sloped to permit of the urine readily draining away. Meissl stated that the collection of the solid excreta uncontaminated by urine was rendered simple by the habit of the pig backing as far as possible from the feeding trough before defecation. The writers, however, have attempted to construct simple metabolism crates on this principle and have never succeeded in securing a satisfactory separation of liquid and solid excreta by this means.

A second type of metabolism crate for swine is described by Forbes (2), who sought in his design to secure comfort and freedom of movement for the animal, free circulation of air and accurate collection of excreta without admixture or contamination. The floor of the crate constituted the special feature. The pig stood on an upper screen below which was a second and lighter screen. The latter was used merely to support a light piece of cloth which retained the faeces falling through the upper screen. Beneath the lower screen was a hopper, by means of which the urine drained away to a collecting vessel. A distinctive feature was the arrangement whereby the upper part of the crate was moveable on small wheels, which ran in grooves in the framework of the lower part. By this means, the upper part containing the pig could be pushed over on to a cleaning table whenever it was necessary either to clean the pig or collect the faeces.

It would appear, however, from the account given by Forbes, that the use of such a crate is not without disadvantages. The arrangements are too elaborate and much care and labour must be expended if satisfactory results are to be obtained. The pig has to lie down on the screen which retains the bulk of solid excreta, and it must therefore be no easy matter to keep the pig clean and secure quantitative collection of the faeces. Furthermore, there must always be considerable risk of contamination of the solid excreta with urine.

After conducting several preliminary trials, the writers came to the conclusion that the problem could not be solved satisfactorily without the use of harness. Kellner, in his work with pigs, was able to devise a satisfactory urine funnel, whilst Lehmann also used in his experiments a "bag and funnel" harness similar to that used in investigations with sheep. This design of harness has, however, always been a source of trouble even when worn by sheep, and it seemed probable that it would prove even more troublesome when employed in work with swine. In investigations carried out with sheep in this institute, the old "bag and funnel" harness has for some years past been replaced very successfully

by the Halnan harness, which has been described in detail in an earlier communication (3). In its essentials, it consists simply of a rubbered canvas sheet attached to the hindquarters of the animal by means of suitable harness. The troublesome collecting bag and the urine funnel are dispensed with altogether. It was decided to modify the design of this harness in certain details to suit the pig's anatomy and to investigate the possibility of employing it in digestion experiments with swine.

I. Description of Harness and Metabolism Crate.

Fig. 1 shows the pig wearing the harness. The rubbered canvas sheet is attached to the hindquarters of the animal by means of leather straps. It is weighted at the lower end in order that it may hang smoothly over a horizontal bar fixed in the back of the crate. By this means, the solid excreta fall directly into a collecting pan stationed in a suitable position on the floor of the metabolism room. No faeces therefore fall on to the floor of the crate.
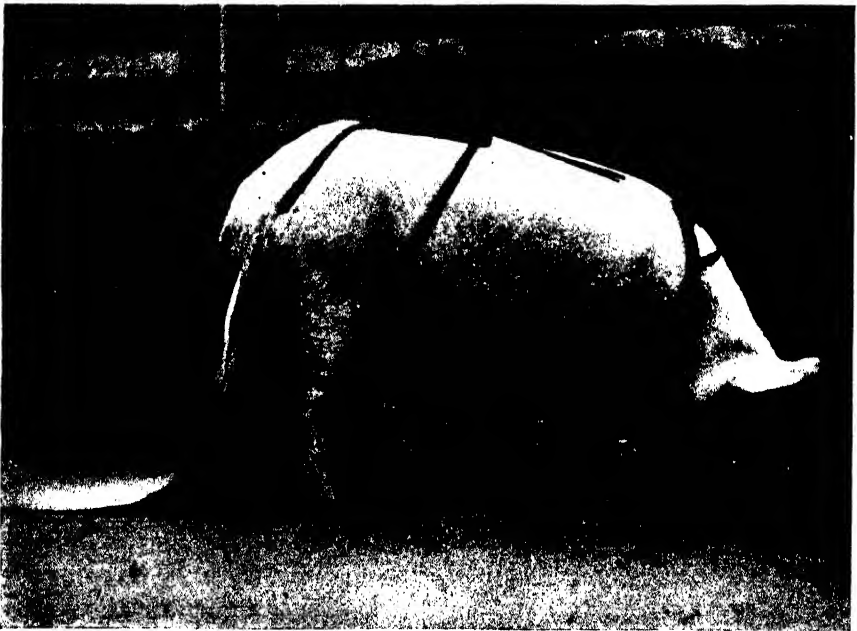
Fig. 1. Showing pig wearing harness.

The harness is so made that it permits of adjustment within wide limits, and it can therefore be used for pigs of varying size. A few inches of strong elastic band are let into the strap which fits along the back of

the animal. This allows for stretching and thus affords greater ease of movement for the animal.

In designing the crate, it was desirable to secure an arrangement whereby the dimensions were capable of easy adjustment to suit the requirements of animals of differing size and weight. For this reason, the back and one side of each cage were so constructed that they could be fixed in any desired position.

The general idea of the arrangements may be gathered from Fig. 2 which represents a simplified plan of a crate designed for use with two animals.
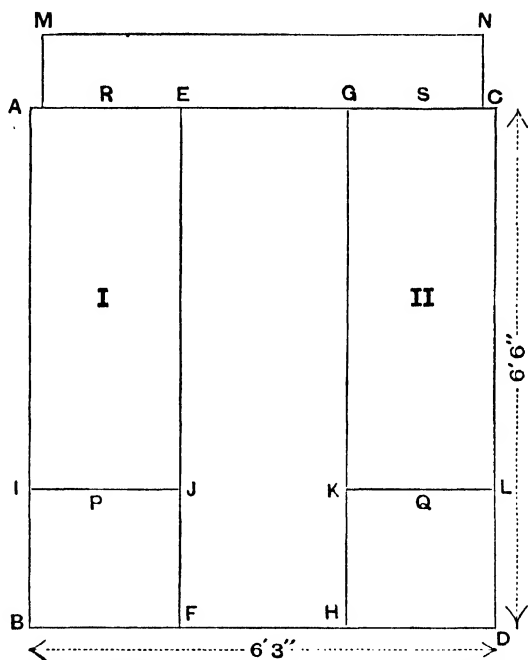
Fig. 2. Representing simplified plan of crate for use with two pigs.

The main framework of the crate was constructed of solid deal beams (3 ins. by 2 ins.), the dimensions being 6 ft. 6 ins. in length, 6 ft. 3 ins. in width and 3 ft. 7 ins. in height. The floors of the cages (I and II) were raised 1 ft. 8 ins. above the ground level. The sides of the cages (AB, EF, GH and CD) were made by drilling holes into the lower and upper deal beams to take 1 inch vertical iron bars. The distance between successive bars was about 3 inches. The two sides AB and CD were joined together by means of horizontal iron straining rods, two at the back and two at the front of the crate. The moveable partitions EF

and *GH* were made to slide along the straining rods and it was possible to clamp them rigidly in such positions that the width of the cages (*AE* and *GC*) was just sufficient to permit the animals to stand up or lie down comfortably, but not ample enough to enable them to turn round. To prevent the animals from having too much freedom longitudinally, moveable frameworks consisting of horizontal iron bars 3 ins. apart (*IJ* and *KL*) could be fitted between the vertical bars of the sides in any desired position. The animals were not given more than about 9 ins. of freedom for movement backwards and forwards.

For the purpose of feeding the animals, lift up doors were made in the front of each cage. The feeding troughs *R* and *S* were bolted down on to a small platform erected outside the cages at the level of the floor of the crate. This made it impossible for the pigs to step into the feeding boxes and the attendant was able to ensure clean consumption of the food.

The floor of the crate consists of stout deal boards 3 ins. in width, placed $\frac{1}{2}$ in. apart and running parallel with the front of the crate *AC*. The boards are triangular in cross section. The upper surfaces which form the floor of the crate have had their edges suitably rounded off, whilst the third edge is kept sharp. In order to avoid rotting of the wood by urine, the boards had previously been soaked for 12 hours in molten paraffin wax. Such an arrangement enables the urine to run off the boards very readily, the liquid dripping from the lower sharp edges into galvanised iron hoppers (3 ft. by 2 ft. 9 ins.) fixed immediately beneath the floor of the cages. The hoppers are so sloped as to enable the urine to drain away into collecting tanks suitably situated at the sides of the crate. All accessible wooden parts of the crate are covered with sheet zinc, with a view to preventing gnawing of the wood by the animals.

During an experiment, the floor boards immediately behind the frameworks *IJ* and *KL* are removed. The rubbered canvas sheets hang smoothly over the lowest bars of the frameworks and the solid excreta thus fall directly into large collecting pans situated on the ground level at *P* and *Q*.

II. Digestibility of Barley Meal.

With the object of testing the suitability of the crate and harness described above, a trial was carried out in which the digestibility of barley meal was determined. For this purpose, a large white hog was selected. Its weight was 286 lbs. at the beginning of the trial. The daily

ration was 2000 gm. barley meal (soaked overnight). The experimental period, during which the solid excreta were weighed and analysed, was of 10 days' duration, this being preceded by a preliminary feeding period of 5 days.

During its stay in the metabolism cage, the animal gave little or no trouble and the arrangements worked very satisfactorily. Similar experiments on other pigs carried out since the date of this preliminary trial have also shown that this method of collecting liquid and solid excreta separately may be recommended with confidence. It is essential to select quiet animals of reasonably clean habits and to accustom them to the conditions before commencing an experiment.

The results obtained in the experiment with barley meal are summarised below:

Analysis of barley meal and composite sample of faeces (calculated on dry matter basis)

	Barley meal %	Faeces %
Crude protein ...	12·29	10·77
Ether extract ...	0·88	7·78
N-free extractives	78·09	42·37
Crude fibre ...	5·58	23·83
Ash	3·16	15·25

The figures for the composition of the barley meal display abnormality in regard to the content of oil, which usually lies between 2 and 3 per cent. Repetitions of the determination, however, confirmed the low value of 0·88 per cent. as representing the amount of ether extract in the sample of meal under experiment.

Mean weight of fresh faeces voided daily = 1186 gm.

Mean weight of dry faeces voided daily = 349·3 gm.

Mean weight of dry barley meal consumed daily = 1672 gm.

Table showing calculation of digestion coefficients for barley meal.

	Total dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Consumed ...	1672·0	1619·2	205·49	·14·71	1305·66	93·30	52·84
Voided ...	349·3	296·0	37·62	27·17	148·0	83·24	53·27
Digested ...	1322·7	1323·2	167·87	—	1157·66	10·06	—
Digestion coefficients %	79·11	81·72	81·69	·—	88·66	10·78	—-

It is seen from the above table that the crude fat constituent displayed a negative digestibility, i.e. the faeces contained more ether soluble material than did the ration of barley meal itself. This, however,

is a disturbance which arises commonly when foodstuffs poor in oil are submitted to digestion trials. Such anomalies are moreover much more likely to occur in experiments with pigs than with sheep, since the results obtained by Kellner (4) show that the disturbing effect of the ether soluble metabolic substances which find their way into the faeces is much more significant with swine than with ruminants. For this reason, the apparent digestibility of the crude fat of a foodstuff is usually appreciably lower with swine than with ruminants, and it is therefore not surprising that in the present instance, with a sample of barley meal so poor in ether extract, the apparent digestibility of the crude fat was negative.

It will further be noted that the utilisation of the inorganic salts of the barley meal was exceptional, since the amount appears to have undergone no diminution during the passage of the food through the alimentary canal.

In the table given below, the digestion coefficients obtained in the present trial are compared with those obtained by Kellner in experiments with ruminants.

	Present trial (pig) %	Kellner's figures (ruminant) (5) %
Organic matter	81·7	81·1
Crude protein	81·7	81·0
N-free extractives	88·7	83·0
Crude fibre	10·8	50·0
Ether extract	Negative	70·0

Excellent agreement is displayed between the figures for organic matter and crude protein. The pig digests the N-free extractives to a somewhat bigger extent than does the ruminant, whereas, as would be anticipated, the ruminant is able to make better use of the crude fibre and crude fat of the barley meal than can the pig.

In the next table is shown the amounts of nutrients digested by the pig (using digestion coefficients obtained in present trial) and the ruminant (using Kellner's digestion coefficients) per 100 parts of *dry matter* of barley meal consumed.

	Pig	Ruminant
Organic matter	79·91	78·18
Crude protein	10·04	9·96
N-free extractives	69·27	64·81
Crude fibre	0·60	2·79
Ether extract	—	0·62

By utilising the ordinary Kellner expression for both pig and ruminant, it follows that the Production Starch Equivalent per 100 lbs. *dry* barley meal is 78·5 for the pig and 77·5 for the ruminant. Whilst it is

unsafe to generalise on the result of one trial, the figures point to the assumption that barley meal possesses approximately an equal productive value for both swine and ruminants, despite the differences which characterise their digestive systems.

During the 15 days of the trial, the pig, which initially weighed 286 lbs., lost 8 lbs. in weight. The mean daily nitrogen balance was + 4·3 gm.

In conclusion the writers have pleasure in acknowledging the valuable assistance of Mr F. J. Aylett in connection with this investigation.

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STUDIES ON THE METABOLISM OF THE RUMINANT BY INDIRECT CALORIMETRY

I

THE INFLUENCE OF VARIATIONS IN THE EXTERNAL TEMPERATURE ON THE ENERGY EXCHANGE OF THE GOAT.

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(With Three Text-figures.)

IN calorimetric work the ideal experimental conditions are that all factors capable of influencing metabolism, except the one whose influence is the object of investigation, should be made constant or otherwise accounted for. It was while striving to attain such conditions that the necessity for this study arose.

During an investigation into the effect of food ingestion on the basal metabolism of the goat by the method of indirect calorimetry described by Orr and Magee [1] it was deemed inadvisable to ignore the diurnal variations in external temperature, and, consequently, it was decided to submit the animal to varying changes of external temperature and to determine their effect upon its basal metabolism. It was the special objective of the experiments to ascertain the critical temperature of the goat or the temperature at which the vital processes of the animal are most economically maintained.

PREVIOUS WORK.

Lavoisier [2] showed that the absorption of oxygen was greater in a resting man at low than at high temperatures. Voit [3] showed that the critical temperature of man lay between 14° C. and 15° C., and Rubner [4] that the critical temperature of the guinea-pig was at 33° C. Rubner also found that the critical temperature of a long-haired dog was between 20° C. and 30° C., and that after being shaved, the dog's metabolism showed a marked increase. Like Voit he believed that the increase in heat production observed at low temperatures was independent of shivering movements, a contention denied by Sjöström [5].

Lefèvre (6) concluded from experiments on different kinds of animals that there is no critical temperature, but that the metabolism decreases steadily as the temperature is raised from 0° C. to the temperature of the animal's body. He also demonstrated the conservative effect of the protective coverings of animals on their heat production. Richet and Segulas concluded that in rabbits and guinea-pigs metabolism is at a maximum between 14° C. and 20° C. Their work has been severely criticised by Lefèvre (*l.c.*).

Leonard Hill and others (7) have demonstrated that the metabolism is raised by cool, out-of-door conditions and that shivering is not a necessary accompaniment of the increased metabolism. The chief factor responsible for the increased metabolism is the cooling power, and not the temperature, of the atmosphere.

The effect of cold food on metabolism is illustrated in an experiment by Lusk (8). When a given amount of glucose dissolved in water at body temperature was ingested, the findings of direct and indirect calorimetry agreed closely; but when the glucose was given in cold water, the heat emission was less than the heat production owing to the necessity of warming the stomach contents.

O'Connor (9) has brought forward evidence to show that the alterations in the blood flow following change in external temperature can be brought about independently by change in the temperature of the brain or of the skin.

Wood and Capstick (10) concluded that the critical temperature of the pig lies round about 21° C.

<center>EXPERIMENTAL PART.</center>

<center>THE ANIMAL.</center>

The animal was an almost fully grown female goat which had lived outside until the beginning of winter. It was in excellent condition, which was maintained during the investigation by adequate daily exercise.

The weight, which was taken daily at the same time, was 29 kilos when the experiments commenced on 6. xi. 22, and 34·5 kilos when the experiments were completed on 16. ii. 23. The gain in weight of 5·5 kilos is chiefly attributable to the fact, discovered afterwards, that the animal was pregnant.

The diet was constant throughout and consisted of:

Foodstuff	Weight in gms.	Calories
Maize	200 ⎫	
Turnips	500 ⎬	2038
Hay	1000 ⎭	

The diet was given daily at noon in one feed. It was believed to be just about sufficient for maintenance, but, as the weight was not a guide, one could not be quite certain that this was so.

The animal was housed in a well ventilated room where the temperature was just about the prevailing shade temperature, and it had acquired, as a result, a fairly good winter coat.

BEHAVIOUR OF THE ANIMAL.

In each experiment recorded all gross muscular movement was absent; otherwise the experiment was abandoned. All the results may, therefore, be taken as pertaining to a condition of minimum muscular activity so far as the skeletal muscles were concerned.

On some cold, still mornings the animal appeared to be stupid and listless; breathing was shallow and slow and the ventilation and metabolic rates were almost invariably lower than one would have expected. The animal brightened up, as a rule, when put into the warm chambers, and the metabolism was higher as a result than in the cold. This listless condition was especially marked from 16. xii. 22 to 16. i. 23 when the weather was damp and depressing.

Polypnoea began when a temperature of 85° F. to 90° F. was reached. Generally, the animal was more comfortable at cold and medium temperatures than at a temperature above 80° F.

EXPERIMENTAL CHAMBERS.

Four small rooms were employed in order to obtain the temperature variations. Their particulars are shown below in tabular form.

Room	Temperatures obtained	Heating	Inspired air	Remarks
A	Outside	Nil.	Pure atmospheric	—
B	48° to 56° F.	Central system	Vitiated by animal during an experiment	—
C	Up to 71° F.	From room D		Anteroom leading to room D
D	Up to or above 100° F.	4 electric radiators		Incubator room

The temperature in room D could be regulated to within ± 1·5° F. The temperatures in any of the rooms during the period the goat was inside rarely varied more than this. A Fahrenheit thermometer was kept constantly in each room, away from sources of heat. The thermometer was read before the animal was put into each room and just after a sampling period was completed. The final temperature was taken as the true reading unless it differed by more than ± 1·5° F. from the initial

temperature, in which case the mean was taken. Wet and dry bulb readings were taken in room A.

The composition of the air inspired by the goat during a sampling period in rooms B, C or D was arrived at by taking the mean of a large number of analyses of the air in each room, before and after the goat had been inside for an experiment. The exact proportion in which the two groups of analyses were to be made was arrived at by taking into consideration the time relations of the several events in the technique. Repeated analyses failed to demonstrate the presence of methane in any of the rooms, in which perceptible currents of air were always guarded against.

DAILY PROCEDURE.

At 8 a.m. the goat was put into room A, and at 8.45 a.m. two samples of expired air were withdrawn, with a two minute interval between them. The animal was then put into room B or C, and after an interval of 45 mins. two more samples were obtained. Finally, the same procedure was carried out after putting the animal into room D.

The collecting periods were 6 mins. long except when the animal was panting, in which case the periods were from 3 to 5 mins. long.

All the samples were measured and analysed the day they were obtained. Altogether nearly 200 experiments were performed.

ESTABLISHMENT OF A BASE LINE.

All the samples of expired air were taken from the animal in the fasting state so that the food factor was inoperative. The temperature could only be fixed in the high temperature room, and, as the fixation of the temperature in this room would have largely defeated the object of the investigation, it was decided to dispense with a fixed base line. It was believed, *a priori*, that the temperature of minimum metabolism was a zone of temperature of a medium degree rather than a single temperature, and that if the metabolism were estimated daily at medium temperatures, the values so obtained would serve as a standard of comparison for those obtained at low and high temperatures.

COMPILATION OF DATA.

When the experimental work was completed, each value for the metabolism was considered in turn and compared with its partner and with the medium temperature values for the same day. Rejections were made on account of departure from the general trend of the results, or of doubtful behaviour on the part of the animal. Such points as the

respiration and ventilation rates and the R.Q. were taken into consideration in deciding if the behaviour was above suspicion.

A considerable number of low values was obtained when the animal was in the listless state already mentioned. The retention of these values for obtaining the mean results meant the averaging of a minority of the results, which were quite comparable amongst themselves, along with the remainder with which they were absolutely incomparable. Thus at 44° F., ten values were obtained whose average value was 35·7 calories per hour, while at the same temperature, four low values were obtained whose average was 28·6 calories per hour. It was obviously unsound to retain the low values. As the condition was regarded as psychic, and as it was very variable in its occurrence, it was decided to reject the corresponding results.
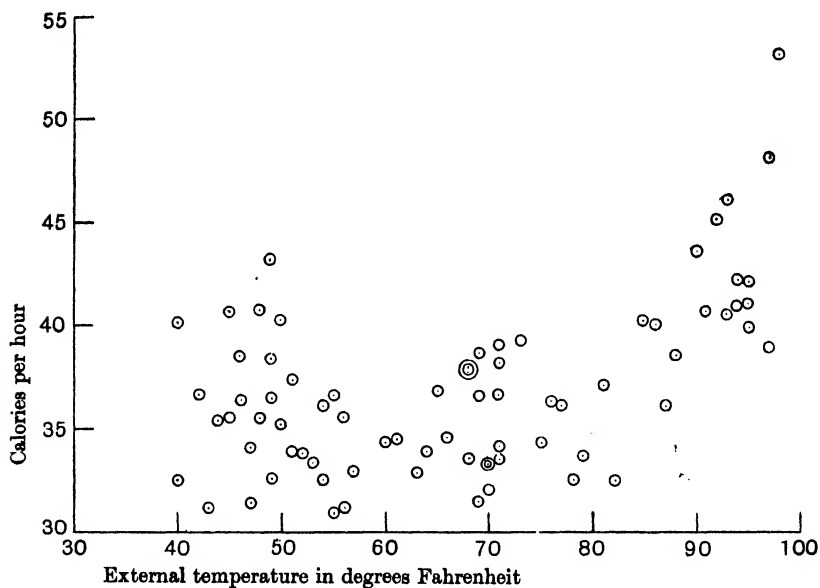
Fig. 1.

Each pair of selected values was then averaged, and the mean values treated as follows:

1. A scatter diagram was plotted (Fig. 1).

2. The temperatures, beginning with the lowest, were tabulated with a range of 2° on each line, and opposite the corresponding values were placed, care being taken to avoid overlapping. To illustrate the method of tabulation, the results between 66° and 72° are shown tabulated below.

Temp. ° F.	Metabolism in calories per hour	Average
66° to 68°	34·6, 31·5⎱	
68° „ 70°	37·8, 36·7, 37·9, 38·7, 33·5⎰	35·8
70° „ 72°	39, 38·2, 34·1, 33·3, 33·5, 33·3, 36·7, 32·1	35·0

The results on each line were then averaged. If on any line there were too few values to form an average, those on two or more lines were averaged together, and the resulting value taken as applicable to the mean temperature. Thus in the above table, the value 35·8 calories per hour is taken to be the average metabolic rate at 68° F.
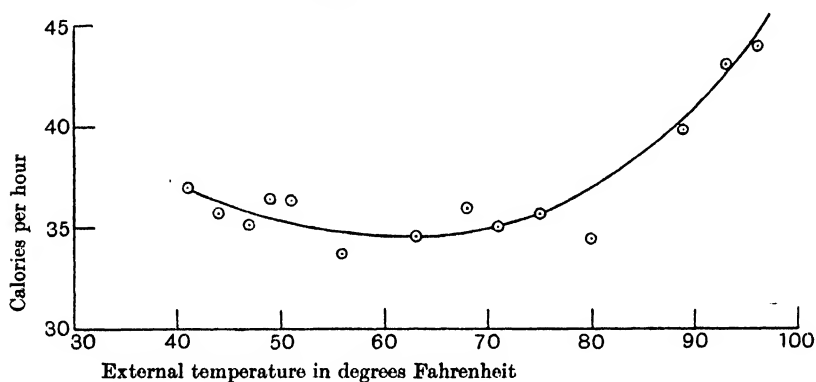
Fig. 2.
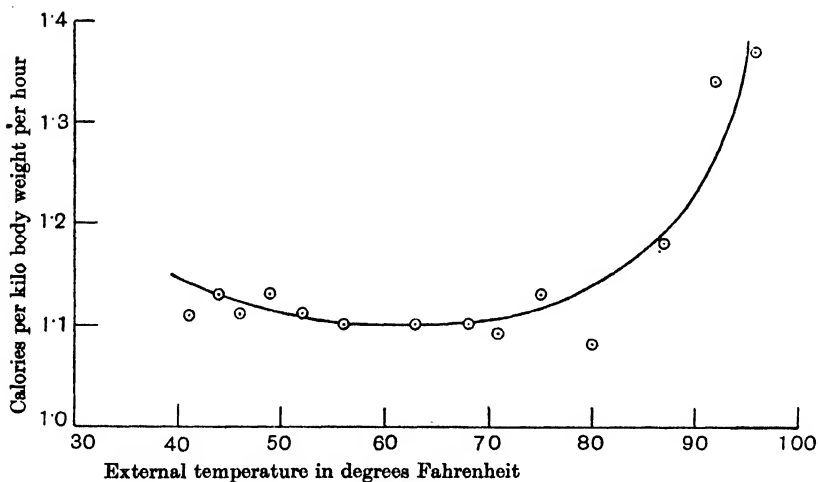
Fig. 3.

A graph (Fig. 2) was plotted showing the metabolism in calories per hour and another (Fig. 3) showing the metabolism per kilo body weight

per hour. The average weekly weight was employed in obtaining the values for the latter graph, which were averaged in the same way as the others. This step was considered necessary on account of the increase in weight during the investigation. It was not possible in either of the above graphs to draw an even curve by joining up all the points; but the curves obtained by drawing as evenly as possible through the points give a very fair approximation of the course of metabolism at the various temperatures.

DISCUSSION OF RESULTS.

Scatter diagram (Fig. 1). The points are very irregular at low and at high temperatures, but less so at medium temperatures. There is, however, evidence of a definite increase in metabolism above 85° and of a slight increase at temperatures below 53° compared with the metabolism at medium temperatures. It is noteworthy that between 51° and 86° there are no points above the line of 39·3 calories per hour.

Calories per hour (Fig. 2). This curve shows that between 55° and 70° the metabolism may be represented practically as a straight line. Below 55° there is a slight, and above 70° a pronounced, gradual increase in metabolism. Taking the value 34·5 calories per hour at 63° as a standard, the increase over this at 44° is 3·8 per cent. and at 96°, 26·9 per cent.

Calories per kilo per hour (Fig. 3). This curve resembles the other fairly closely except that the points are on the whole more even. Taking the value 1·10 calories at 63° as a standard, the increase above it at 44° is 2·7 per cent. and at 96°, 24·5 per cent. The increase is, therefore, less conspicuous when reckoned per unit of weight. This curve, in which the weight factor is accounted for, gives a better representation of the course of metabolism at the different temperatures than the other. The curve from about 55° to about 70° is a straight line, and as the metabolism rises at temperatures above and below these limits, it may be concluded that the critical range of temperature is from 55° to 70° F.

THE R.Q.

The R.Q.'s, when averaged for every 10° F., beginning with the lowest temperatures, were:

From 38° to 50° and from 50° to 60°	...	...	·82
„ 60° „ 70° „ 70° „ 80°	...	...	·81
„ 80° „ 90° „ 90° „ 98°	...	...	·83

The increased metabolism at temperatures beyond the critical zone was evidently brought about by a relatively greater combustion of carbo-

hydrate than what occurred within the critical zone. Freund and Marchand[11] found that the blood sugar in rabbits was at a minimum at external temperatures of from 30° to 34° C. The changes in blood sugar might be explained as Barbour[12] suggests, by dilution or concentration of the blood. Freund and Marchand conclude that the rise in blood sugar obtained at low temperatures has no relation to increased combustion of carbohydrates; but they do not state whether this occurs at low temperatures.

After comparing all the results at the cold temperatures, it was found that when the animal fell into the drowsy condition a low temperature was generally associated with a high moisture content of the atmosphere; but very often when these atmospheric conditions prevailed, the mental state of the animal appeared as bright as usual, and a high rate of metabolism was obtained.

DISCUSSION.

There are no known records of experiments performed to determine the effect of changes in the environmental temperature on the heat production in ruminants. Bull[13] states that the fermentation of the food in these animals produces enough heat for the maintenance of the body temperature in cold surroundings, while Armsby[14] believed that their critical temperature is low, owing to the large amount of heat arising from the work of digestion. The results of this investigation go to prove the falsity of Bull's contention, while the theory that the work of digestion occasions a large heat production is no longer tenable (*v.* Lusk[15] and others).

The zone of critical temperature found was from 55° to 70° F., *i.e.*, from 12·8° to 21·1° C. The lower limit is almost the same as the critical temperature for man as found by Voit (*l.c.*), and the upper limit is identical with that found for the pig by Wood and Capstick (*l.c.*). The range of temperature, however, is only a little less than that found by Rubner (*l.c.*), viz. 10° C., for a long haired dog. It is evident that the effect of a good protective covering for the skin is to extend the range of the critical temperature.

The above findings go to strengthen the contention of Rubner and Voit that chemical regulation of temperature is independent of shivering movements, for the goat was never observed to shiver in the cold.

In connection with the experiments, it is necessary to bear in mind the following particulars regarding the animal.

(*a*) The animal was housed in an environment at a temperature about the prevailing shade temperature. Cool, outside conditions entail a higher

basal metabolism than warm indoor surroundings (L. Hill and others) (*l.c.*). It seems very probable, therefore, that if the goat had been housed in a medium temperature, a greater metabolic response than that found would have been induced by cold.

(*b*) The animal's dietary was just about sufficient for its requirements. As Rubner has shown (16), the effect of food on the heat regulatory mechanism is to lower the critical temperature towards zero, and the more protein the food contains the greater is its effect in this regard. It may be inferred, therefore, that the critical zone as found above would only hold good for a maintenance diet of similar composition.

(*c*) The technique employed entailed the persistence in a minimised form of visual, auditory and other external stimuli, which, though they could not be entirely abolished, were rendered fairly constant by routine practice. The experimental conditions in this respect differed from those obtained by most other observers in this field by the use of chamber methods. These methods undoubtedly record a metabolism that is the nearest possible approach to the theoretical basal metabolism, but it is a moot point whether their results are, in a comparative sense, of any more value than those obtained by the Douglas-Haldane method on an animal submitted to exactly the same procedure day after day.

The estimation of the critical temperature of the domesticated animal under conditions as near the natural state as possible and on a maintenance diet is of greater practical interest than if conditions are introduced such as artificially heated surroundings, or starvation, that have no parallel in the ordinary life of the animal. As the former conditions prevailed, it is believed that the results of these experiments should be of some value for the stock farmer.

The onset of a drowsy condition in cold, damp, depressing weather appears to be a provision of nature whereby an animal preserves its energy by limiting its dissipation. Illustrative examples are numerous in nature, and it may be that every animal possesses in a more or less marked degree, depending on its position in the phylogenetic tree, latent hibernating powers that are called into action only in times of stress. Russian peasants, in famine years, have been able to weather the winter with little or no means of subsistence by passing the time in almost uninterrupted sleep huddled together near the stove, thus conserving their resources (Morgulis (17)). A further example is the common experience of the farmer who goes to see his cattle in the field on a cold depressing morning. He finds them sometimes huddled together with backs humped and difficult to arouse, and yet he is aware of the risk he would run by

setting free his stall fed animals on such a morning, a risk which is much less on a mild morning. The differences in behaviour appear to be due to the fact that the indoor animal is well fed and so has no necessity to husband its reserves, and is also acclimatised to a moderate temperature with an ill-developed coat of hair.

It is clear that the less oxidisable material an animal has to draw upon, the more economically does it strive to live. Therefore the longer the homeothermic animal is deprived of food, the more nearly will its critical temperature approach the body temperature. Lefèvre's (*l.c.*) contention that the temperature of minimal metabolism is identical with the body temperature of the animal appears less disputable in the light of these considerations.

CONCLUSIONS.

The critical range of temperature for the goat acclimatised to the shade temperature in winter and on a maintenance diet is from 55° to 70° F. As the temperature falls below 55°, metabolism exhibits a slight gradually increasing rise, due to the oxidations necessary to cope with the increasing heat loss; and, owing to the gradually increasing efforts of the animal to promote heat dissipation by panting, metabolism shows a pronounced gradual increase as the temperature rises above 70° F.

Cold, damp and depressing weather has occasionally a psychic effect on the animal which falls, at times, into a torpid condition with flaccid muscles, shallow respirations and a low rate of metabolism.

It is a pleasure to record my indebtedness to Dr J. B. Orr, Director of the Institute, for instruction in the technique, for his suggestion of the research and for his kindly criticism and advice.

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STUDIES ON THE METABOLISM OF THE RUMINANT BY INDIRECT CALORIMETRY.

II

THE INFLUENCE OF PREGNANCY ON THE ENERGY EXCHANGE OF THE GOAT.

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(With Five Text-figures.)

THIS paper is founded on the basal metabolism values obtained during investigations on the influence of variations in the external temperature and of food on the metabolism of the goat and in addition on the basal values obtained in an independent investigation. When, late in pregnancy, the animal's condition was diagnosed it was decided, owing to the absence of any reference in the literature to the effect of pregnancy on the total energy exchange of the ruminant, to collect all the available data for this animal and to continue to determine its basal metabolism daily with the object of securing a record of the course of metabolism during pregnancy and the puerperium.

PREVIOUS WORK.

There are very few records in the literature of experiments whose objective was the determination of the effect of pregnancy on the total energy exchange. The most complete study of this aspect of pregnancy is due to Magnus-Levy [1], who showed that the oxygen consumption increased in a pregnant woman from month to month. He found an increase of 27 per cent. in the oxygen consumption in the 9th month over that of the same woman non-pregnant. The increase in oxygen consumption was first noted in the 3rd month. Murlin [2] found that the increase in metabolism due to pregnancy in dogs is proportional to the weight of the offspring and also [3], that the metabolism per unit of weight was higher in women soon after, than just before, delivery. Cornell [4] determined the basal metabolism in the last month of pregnancy and

from 3 to 16 days *post partum* in 84 women and found an average increase of 29 per cent. over the non-pregnant rate before, and of 21·5 per cent. after, parturition. He quotes Baer who found an increase before delivery of 33 to 35 per cent. over the non-pregnant metabolic rate and of 15 per cent. soon after delivery. Baer concluded that the metabolism returned to normal 7 to 10 days *post partum*. Rowe and Eakin(5) found that metabolism rises in the last months of pregnancy.

EXPERIMENTAL PART.

The Animal.

The animal gave birth to a single kid on 27. iii. 23, and the commencement of pregnancy was reckoned as 22. x. 22, by counting back 22 weeks, the gestation period of goats, from the date of parturition. The animal was a primipara. The kid was removed from its mother after birth and the goat was milked three times daily for a while, and later, twice daily. In each experiment recorded, the animal was in a condition of minimum functional activity, *i.e.* all gross bodily movement was absent. The animal was kept in good condition by daily exercise.

The Diet.

From 13. xi. 22 to 20. ii. 23, a mixed diet of 2038 calories was given daily in one feed at noon and from 21. ii. 23 to 28. iii. 23, a mixed diet of 2386 calories was given in two portions at 9 a.m. and 4.45 p.m. Both diets were closely similar in nature. After delivery the diet was decreased by 344 calories owing to diminished appetite.

During and between the sampling periods, the animal was kept in the same experimental room in the basement area on the north side of the building throughout the period of investigation. From the results of previous experiments(6) it was believed that the variations in external temperature could not, of themselves, cause changes in the metabolism of such magnitude as to invalidate any conclusions one might draw from the results.

The Experiments.

The technique employed has been described by Orr and Magee(7). The sampling periods were from 6 to 10 mins. long. Each working day, two or more estimations were made at least 15 hours after the last food. The weight was taken each morning before experiments were begun.

Experiments were started at the beginning of the 4th week of pregnancy and continued until four days before parturition. Estimations

were continued after delivery for five weeks. Unfortunately, it was not possible to obtain a complete series of results, but the data obtained, form, on the whole, a fairly complete record of the course of metabolism during pregnancy.

COMPILATION OF DATA.

The gestation and *post partum* periods were divided up into weeks and tabulated. Opposite each week were placed the corresponding results which were then averaged after discarding doubtful values. Thus, there were obtained for each week, the average tissue heat, R.Q., fermentation heat and weight. Curves were then plotted with the age of pregnancy in weeks as abscissae and these weekly average values as ordinates.

DISCUSSION OF RESULTS.

The Weight (Fig. 1). The weight increases gradually from a value of 29·4 kilos in the 4th week to one of 39·5 kilos in the 22nd week, an
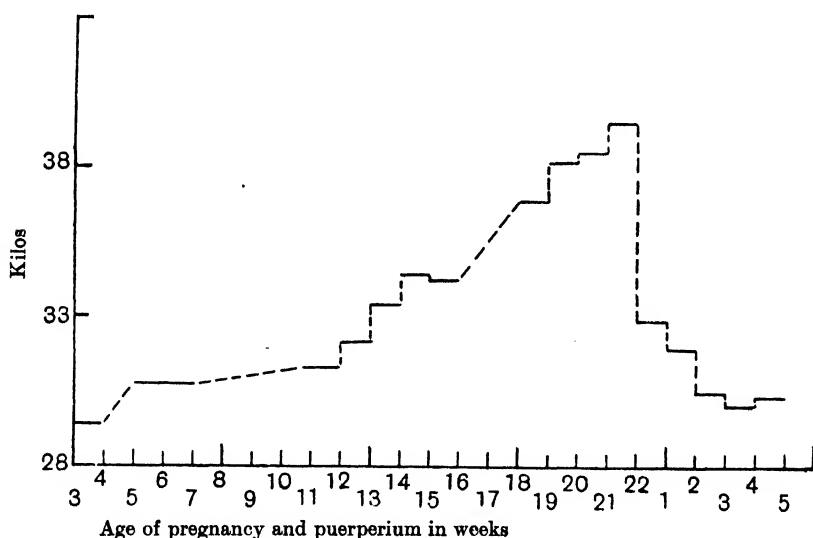
Fig. 1. Weight.

increase of 10·1 kilos. In the 1st week after parturition it was 32·9 kilos, *i.e.* a drop of 6·6 kilos. It became steady in the 3rd week.

Metabolism (Fig. 2). The tissue heat remains fairly steady until the 13th week, when the rate is 34·0 calories per hour. In the 14th week it has increased to 38·6 calories per hour. From the 14th week, metabolism continues to mount up until a maximum of 51·2 calories per hour

is reached in the 21st week, *i.e.* an increase of 50·6 per cent. over the average for the 13th week. In the 22nd week there is a slight fall to 50·4 calories per hour and a big fall to 45·6 calories in the 1st week after delivery, *i.e.* a drop of 8·9 per cent. below the rate in the 22nd week.
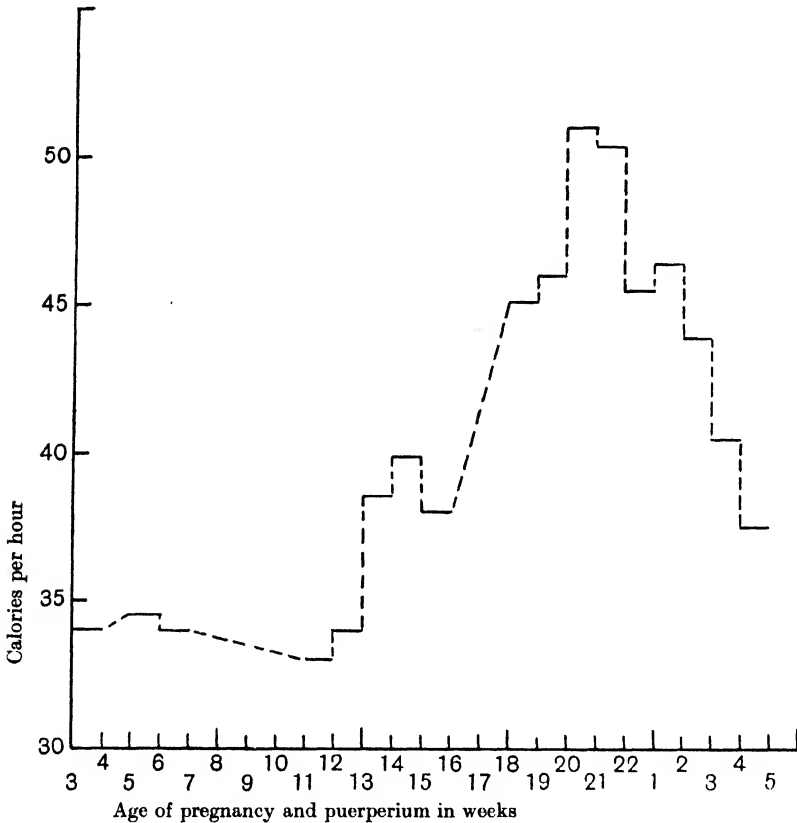
Fig. 2. Total metabolism.

After an insignificant increase in the 2nd week, metabolism falls fairly regularly to a value of 37·7 calories per hour in the 5th week *post partum* when the experiments were terminated.

Metabolism per kilo body weight (Fig. 3). In the 4th week the value is 1·16 calories per hour, then there is a drop, which one may assume is gradual, to a value of 1·06 calories in the 12th and 13th weeks. These facts seem to indicate that the tissues of the mother are stimulated to increased activity by the young ovum soon after impregnation and that metabolism begins to fall off in intensity for a time as pregnancy advances.

The early increase in metabolism soon after impregnation of the ovum is interesting in view of the increased protein catabolism in early pregnancy as evidenced by a negative nitrogen balance. From the 14th week there is a steady upward rise with oscillations to a maximum value of 1·45 calories per hour in the 2nd week after delivery, *i.e.* an increase of 36·8 per cent. over the rate in the 13th week. The rate in the 21st week, 1·33 calories,
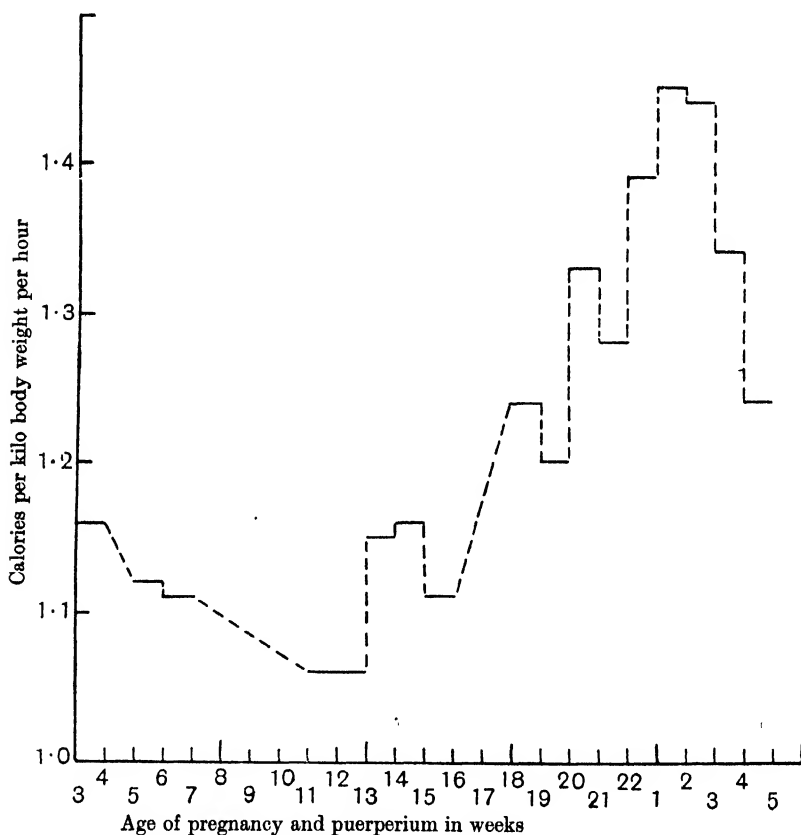
Fig. 3. Metabolism per kilo.

is 25·4 per cent. above that in the 13th week. The corresponding incre-ment in the total metabolism is 50·6 per cent., so that one might conclude that the increase of 50·6 per cent. in the total metabolism is half due to the increase in the mass of active protoplasm and half due to the stimulus of the products of conception on the mother's tissues. The curve shows that the stimulus becomes intensified from the 14th week onwards. The high values in the first 3 weeks after parturition can have no other

meaning than that the involution of the uterus and the other reparative processes that follow delivery lead to increased activity of the active protoplasm. This interpretation is to this extent in conformity with that of Murlin (8) for the similar phenomenon observed in three women, but the big drops in the 4th and 5th weeks are against his contention that lactation is in part a cause of the increased metabolism, for secretion of milk increased instead of diminishing in the first few weeks after delivery.

The R.Q. (Fig. 4). Until the 14th week the R.Q. is so irregular that definite conclusions cannot be drawn from it; but there is a very slight downward tendency from the 16th to the 22nd week. There is a decided drop from a value of ·81 in the 22nd week to one of ·71 in the 2nd and 3rd weeks after delivery. It then begins to rise again, but in the 5th week
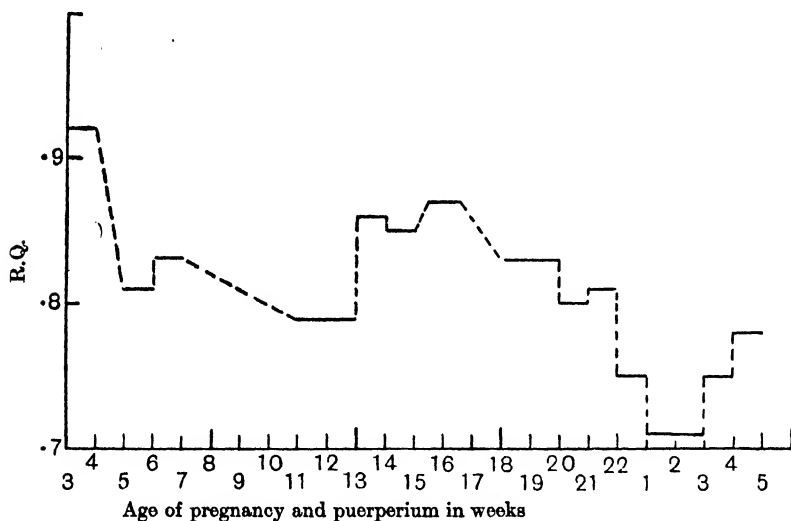
Fig. 4. Respiratory quotient.

it is still lower than any value obtained in the *ante partum* period. One may therefore conclude that the R.Q. is higher in the pregnant goat than in the same animal in the early weeks after delivery. Murlin (9), from experiments carried out by himself and Carpenter, concluded that the R.Q. is higher just before parturition than just after, the reason for which he suggests is the limited dietary permitted the human mother soon after delivery.

The Fermentation Heat (Fig. 5). It is believed that the fermentation heat rate cannot be regarded in the fasting animal as an index of the rate of fermentation, since the calculation is based on the excretion of

methane which is effected at irregular intervals. If one looks upon the fermentation heat rate simply as the rate of excretion of fermentation gases, it affords an index of the amounts of these gases in the rumen at the time of the experiment from which can be deduced the relative extent to which fermentation proceeds in the rumen of the animal in different functional states.
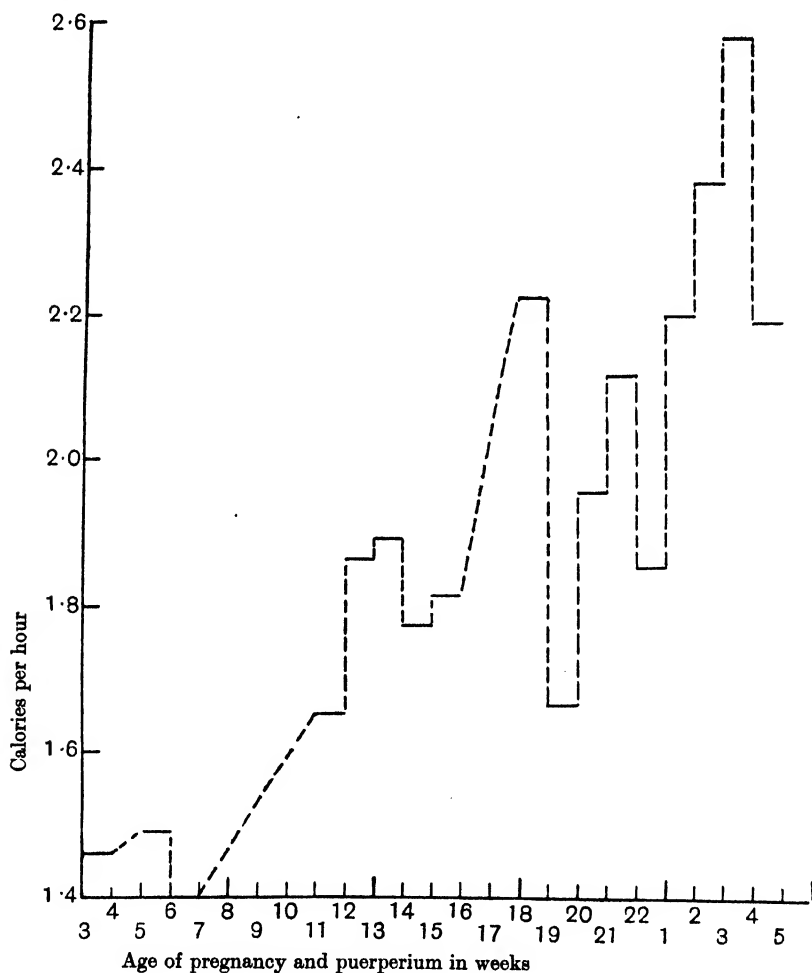
Fig. 5. Fermentation. Heat.

The curve shows an upward tendency from the 4th to the 22nd week with wide and irregular oscillations. After a temporary drop in the 1st week after delivery, explainable by the fact that the animal's appetite

became impaired, the rate of gaseous excretion rises steadily to a value at the 4th week far above any obtained during pregnancy. Then there is a drop in the 5th week, but the rate is still well above all the rates during pregnancy except that in the 21st week with which it is just about equal.

These facts are interpreted as follows:

Soon after pregnancy begins the intra-abdominal pressure increases, thus compelling the earlier evacuation of the rumen in the pregnant than in the non-pregnant animal. Fermentation is thus cut short. As pregnancy advances the greatly increasing pressure partially impedes the evacuation of the rumen, so that fermentation is relatively more complete than in early pregnancy. The low pressure ensuing after delivery accentuated by the lowered tone of the abdominal walls allows the food to remain a longer time in the rumen, so that fermentation and gas production are more extensive than during pregnancy.

The curtailment of fermentation in the pregnant animal leads to the oxidation of a greater proportion of metabolites from unfermented food than in the non-pregnant animal. From later experiments (unpublished) it is believed that the oxidation of the fermentation products of all foods and even of carbohydrates gives a R.Q. much below ·80. Therefore, the more extensive the fermentation, the lower will be the R.Q. Hence, we have a reason for the slight falling off in the value of the R.Q. with the advance of pregnancy and for the big drop that occurs after parturition. The fact that there was a slight rise in the R.Q. in the 4th week *post partum* when the fermentation heat was at a maximum does not at first sight conform with this interpretation. It is possible, however, that the R.Q. in the 2nd and 3rd weeks is abnormally low owing to conversion of the nitrogenous involution products into sugar, for Lusk (10) has shown that a low R.Q. is obtained during the retention in the body of carbon from protein for conversion into carbohydrate.

SUMMARY.

The total metabolism of the pregnant goat remains at a steady rate from the 4th to the end of the 13th week of pregnancy. It increases steadily from the 14th to the 21st week, when a value is attained about 50 per cent. above the rate at the 14th week. Just before delivery the rate drops slightly. The increased metabolism is due partly to the increase in the mass of active protoplasm and partly to the stimulus exerted by the growing ovum on the maternal tissues. This stimulus tends to fall

off slightly in intensity in early pregnancy and increases markedly in late pregnancy.

Post partum the total metabolism drops suddenly and continues to fall until the end of the 5th week; but the metabolism per unit of weight increases steadily until the 4th week and then declines. This increased activity of the maternal protoplasm is due to the processes of involution and repair.

In early pregnancy the increasing intra-abdominal pressure cuts short fermentation of food and causes the R.Q. to be higher in the pregnant than in the non-pregnant animal. As the pressure increases it begins to impede slightly the evacuation of the rumen so that the food ferments more thoroughly and the R.Q. falls slightly. After parturition the low intra-abdominal pressure aggravated by the laxity of the abdominal walls allows the food to remain longer in the rumen so that fermentation is more thorough and the R.Q. lower than in late pregnancy.

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STUDIES ON THE METABOLISM OF THE RUMINANT BY INDIRECT CALORIMETRY.

III

THE INFLUENCE OF WORK ON THE ENERGY EXCHANGE OF THE GOAT.

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Two objectives were in view in undertaking this research; to ascertain the cost in energy of muscular work in the goat and to investigate the adaptability of the Douglas-Haldane method of indirect calorimetry to work experiments on this animal. It is to the results of human experiments chiefly, that the present position of the physiology of muscular exercise is due which owes comparatively little to experiments on other animals. Zuntz and his school [1] have, however, compared the effect of a fixed amount of work on men, horses and dogs, and Lusk [2] has carried out some investigations into the influence of work on the dog. The principal obstacle hitherto in the way of employing animals other than man for work investigations has been the want of a sufficiently adaptable technique. Zuntz's experiments on the horse were conducted by means of a tracheal cannula, a device that, though fairly adaptable for work experiments, is not always desirable. Lusk's experiments were carried out in his respiration calorimeter where the applicability was very limited. Boothby and Sandiford [3] and Kunde [4] have carried out metabolic investigations on dogs by means of the Benedict apparatus, but here again the device is only of limited value for work experiments, which none of these workers have apparently yet undertaken. It was believed therefore that, if the proposed experiments were a success, in addition to possessing a certain physiological value, they would open up a wide field of research of great practical significance.

EXPERIMENTAL PART.

THE ANIMAL.

The animal was given a mixed diet of liberal proportions in two portions daily, at 10 a.m. and at 4.45 p.m. The diet was the same during the research and for 10 days before it was started. The weight remained steady throughout. Training was commenced 6 weeks before the experiments were begun and was carried out at first once, and later twice, daily.

THE WORK.

The work consisted in walking at two rates along the hard, smooth and level walk round the building, a circuit of 147 yards. The rates of walking were:

Slow, $1\frac{1}{2}$ minutes for the round = 98 yards per minute.

Fast, $1\frac{1}{4}$ minutes for the round = 117·6 yards per minute.

The mask used in these experiments caused some distress at more rapid rates than the fast rate of walking and it was difficult to induce the goat to travel very slowly, hence the nearness of the rates of walking. Except for occasional attempts to stop or to go too fast the behaviour of the animal was satisfactory during the investigation.

Except on two days the weather was mild with only a gentle wind blowing.

THE EXPERIMENTS.

The application of the Douglas-Haldane method of indirect calorimetry to the goat has already been described (Orr and Magee (5)). The experiments were all carried out before 10 a.m. under "basal" conditions. At 8.30 a.m. two basal samples of 10 mins. each were collected with the goat standing quietly and at rest and immediately afterwards it was walked twice round the building at the slow rate. The mask and bag were quickly applied to the animal and tested carefully for leakage. Another circuit of the building was completed and then the tap of the bag was turned. The expired air was collected for 2 mins. Then the bag was unstrapped and a fresh one rapidly substituted. The goat was walked another $\frac{3}{4}$ lap at the slow speed, then the tap was turned and another 2-minute sample obtained. The four bags of air were then measured and samples obtained from each during which time the goat was resting in a quiet place. Then two samples at the fast rate were obtained as above. These were measured, samples taken from each, and all six samples analysed.

About 20 results in each series (resting, slow and fast rates) were obtained. These were tabulated as below and averaged, use being made of the principles laid down by Gephart, Du Bois and Lusk (6).

Metabolism in calories per hour, standing		Metabolism in calories per hour, slow rate		Metabolism in calories per hour, fast rate	
48·0	52·7	252·5	263·6	307·0	—
57·6	45·4	268·2	276·5	282·1	304·1
50·9	—	251·2	288·7*	271·2	305·1
52·6	—	254·2	236·2	295·9	299·8
45·7	45·3	245·2	253·3	293·4	318·0
46·6	47·3	243·1	251·4	275·2	321·9
49·0	44·1	253·2	199·1*	251·5	287·0
44·5	44·4	235·1	243·7	293·0	307·2
45·3	43·4	205·3*	217·9*	287·4	283·0
47·3	—	244·9	243·2	276·1	293·3
41·3	43·6	257·6	222·7	262·3	264·1

Average 45·7   249·8   291·2

* =rejection.

The values for the R.Q. and fermentation heat were compiled similarly, except that the above method of averaging, being superfluous, was dispensed with in the case of the R.Q.; and as the fermentation heat values were extremely variable the principles could not be applied to them.

DISCUSSION OF RESULTS.

Tissue Heat. The average values for the tissue heat are:

Rest: 45·7 calories per hour.

Slow rate: 249·8 calories per hour, increase due to work = 204·1 calories.
$$= 446·6 \text{ per cent.}$$

Fast rate: 291·2 calories per hour, increase due to work = 245·5 calories.
$$= 537·2 \text{ per cent.}$$

$$\frac{\text{Fast rate}}{\text{Slow rate}} = \frac{117·6}{98} = 1·2.$$

$$\frac{\text{Excess metabolism due to work (fast)}}{\text{Excess metabolism due to work (slow)}} = \frac{245·5}{204·1} = 1·2.$$

Therefore, at the above rates of progression, the excess metabolism due to a fixed amount of work varies directly as the rate at which it is performed. In other words the cost of moving a given mass through unit distance at the rates studied is the same.

Cathcart *et alia* (7) came to the conclusion that a sufficiency of individual experiments over a sufficiently wide range of speeds have not been carried out to enable one to express in quantitative form a general physiological law governing the relation between work and the rate of

its performance. Cathcart and Orr (8) have criticised the work of Zuntz and Schumburg who put forward the view that the cost of performing a fixed amount of work increased directly with the velocity of performance. It is to be inferred, however, from the discussion of the former observers (p. 56) that there is an optimum economical rate of progression which they found for one subject under constant load to be 88·38 yards per minute. This view is in accordance with that of Frentzel and Reach arrived at from work carried out in Zuntz's laboratory.

In the present investigation since the costs of moving the same mass of body substance through unit distance at 98 and 117·6 yards per minute were found to be the same, it might be concluded that the optimum rate of progression for the goat lies somewhere between these rates.

It is interesting to compare the cost of muscular work in man and in the goat. Cathcart and Orr (*l.c.*) found that the average rate of metabolism for subject *M* "standing at ease" was 74·1 calories per hour (p. 15). The rates for the same subject marching with a load of 5 kilos were, at 100 yards per minute 318 calories, and at 120 yards per minute 415·8 calories per hour (pp. 45 and 46), *i.e.* an increase of 329 per cent. and 461 per cent. respectively over the standing metabolism. These rates of marching approximate closely to those employed in the present study and for this reason the two groups of results can be compared. Below, a comparison is made in tabular form.

Rate of marching	Percentage increase over standing metabolism
	Man
100 yards per minute	329
120 ,,	461
	Goat
98 yards per minute	447
117·6 ,,	537

Evidently, then, the cost of progression at similar rates is more costly in the quadruped than in the biped. This can be accounted for in one or other or both of the following ways:

(1) The quadruped brings into action a relatively greater mass of muscular tissue than does the biped in walking.

(2) The biped has a relatively greater expenditure of energy in the standing position than has the quadruped on account of its less secure base of support. If this were so it would make the increment due to work appear relatively less than in the case of the quadruped, when comparison is made with the standing metabolism. To settle the question definitely it would be essential to compare the metabolism in both types

of animals in the lying and standing positions and during work. Unfortunately, no data were obtained for the goat used in this research in the lying position; but results have been published (Orr and Magee, *l.c.*) for another animal which go to prove that the cost of standing is similar in the biped and quadruped. One may conclude, therefore, that the relatively greater cost of walking in the goat as compared with that in man is due to the operation of a correspondingly greater mass of muscular tissue.

The R.Q.

The average R.Q.'s are:

Rest	Slow rate	Fast rate
·78	·82	·84

These figures show that the R.Q. rises with increase in the velocity of performance of a fixed amount of work. Krogh and Lindhard (9) have shown that on transition from rest to work a resting R.Q. below ·80 is raised and one above ·90 is lowered while one between ·80 and ·90 is not altered. Hill and Lupton (10) state that moderate and steady work results in a R.Q. approaching unity while severe work gives a R.Q. above unity.

The above results seem to fall into line with the findings of both groups of workers; but as their experiments were directed towards a solution of the mechanism of cell energetics and as their explanations of the phenomena are different, further discussion is irrelevant.

The Fermentation Heat.

The average fermentation heat values are in calories per hour,

Rest	Slow rate	Fast rate
2·55	4·04	3·61

The figures for the work experiments were extremely variable. The maximum and minimum values were, slow rate 10·64 and 0·90 calories per hour respectively, fast rate 9·17 and 1·03 calories. All the values were averaged indiscriminately. The wide variations are undoubtedly due to the effects of the exertion and to the fact that the collecting periods were of very short duration, 2 minutes. It has been observed repeatedly that the shorter the periods of sampling the more uneven will be the estimated rates of excretion of fermentation gases of which the calculated fermentation heat is a measure and not, in a strict sense, of the rate of fermentation in the rumen. It is therefore believed that the above averages are approximately a measure of the rates of gaseous

excretion in the three functional states of the animal. They show that exercise increases the rate of gaseous excretion from the rumen, which, as the work is prolonged, begins to decline owing to the relatively large amounts of gas eructated in the early stages of the work. It is possible, too, that the exertion leads to evacuation of food from the rumen and thus cuts off the source of the gases soon after commencement of the exercise.

CONCLUSIONS.

1. The cost of forward progression in the goat is relatively greater than in man because a relatively greater mass of muscle is brought into play by the former in the movements associated with walking.

2. Work raises a previously low resting R.Q. and increase in the rate of work raises it still higher.

3. Work causes an increase in the rate of excretion of fermentation gases which declines as the work is prolonged.

4. The Douglas-Haldane method of indirect calorimetry is a reliable means of estimating the energy expenditure of the goat during muscular work.

My sincere thanks are due to Dr J. B. Orr, Director of the Institute, for advice and suggestions.

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THE INFLUENCE OF THE ADMINISTRATION OF CERTAIN OILS ON THE NUTRITIVE VALUE OF THE BUTTER FAT OF COWS ON WINTER RATIONS.

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(With Seven Text-figures.)

THE work described in this paper was planned in order to obtain further and more exact information than had been obtained in an experiment carried out during the previous year on the same subject and reported in this journal by Drummond, Coward, Golding, Mackintosh and Zilva (1923). In this paper, additional confirmation was presented in support of the relationship between the presence of vitamin A in the milk and its supply in the food, and it was shown that the characteristic decline in the concentration of vitamin A in the milk fat which tends to occur during the winter feeding of cows on the customary ration of roots, hay and concentrates may be prevented by the administration of relatively small doses of cod liver oil. As stated in that communication, further experiments appeared to be desirable. One point open to criticism was that the samples of butter examined were made from the mixed milks of groups of cows. An illustration of how this could lead to a disturbance of the course of the experiment was described in that paper, when it was shown that the decline of the vitamin A concentration of the mixed milk of the groups was not only arrested, but temporarily converted into a

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sharp rise, by the inclusion in one group at that stage of the test of an animal which had recently calved. It was with this and other points in view, that we decided to carry out a similar experiment, in which the butter made from the milk of individual cows, was to be studied. In this way an explanation of any individual result which differed materially from the general result, might be sought for in the past history, etc., of the animal concerned. There can be no question that the best manner to carry out such an experiment as this is to test the milks rather than the butters. Unfortunately, the difficulties of ensuring fresh supplies of milk from Reading, where the feeding experiments were made, to the laboratory at University College, where the vitamin A testing was done, were so great, that after careful consideration it was decided to examine the butters instead. In the new experiment, it was decided to make the control of the composition of the rations of the cows very much more complete than had been done in the previous year, particularly as it was intended to make the experiment serve not only to provide information on the practical question of cod liver oil administration to milch cows, but also to yield evidence regarding the chemical composition of butter fat from cows on different diets. For this reason, a basal ration of roots, hay and concentrates was chosen so as to be not only as free from vitamin A as possible, but also of very low fat content.

DETAILS OF THE COWS USED.

The cows chosen for the experiment were dairy shorthorns selected from the herd of the National Institute for Research in Dairying at Reading.

Table I.

	No. of calf	Last calved	Condition
Lily	1	17. xi. 22	Fairly good
Lucy	1	4. xii. 22	Good
Scarlet	3	8. xi. 22	,,
Fillpail II	7	5. i. 23	Fairly good

All the cows had recently calved; hence it was probable in the light of the previous year's experience that any effect due to storage of vitamin A by the animals, would be similar in all cases. The cows were placed in well-lighted stalls at various times between the middle of December and the beginning of January and were given the basal diet up to February 26th.

COMPOSITION OF THE BASAL DIET.

The basal diet consisted of mangolds, hay and concentrates, the latter made up of equal parts of maize gluten feed, crushed wheat and soya bean meal, the last having been commercially fat-extracted. The daily ration of each cow varied little from

Mangolds	...	...	...	60 lbs.
Hay	...	...	...	15 „
Concentrates	...	...	...	9 „

Full details of this are given in Table II.

(1) FAT INTAKE ON THE BASAL DIET.

The percentage of ether-soluble matter in the foods was:

	% dry weight
Mangold	0·1
Hay	2·2
Maize gluten feed	2·2
Soya bean meal	1·2
Crushed wheat	1·7

A half day's ration after careful drying and powdering was fat-extracted in a large Soxhlet extractor and yielded 66 gms. of heavy green oil having a saponification value of 169·5, iodine value 118·4, and containing 18·75 per cent. unsaponifiable matter. This would give an average daily intake of ether-soluble matter of approximately 132 gms. of which just over 100 gms. or about 4 ozs. can be regarded as saponifiable matter.

(2) VITAMIN A CONTENT OF THE CONSTITUENTS OF THE DIET.

(a) *Mangolds.* Steenbock and Gross (1919) reported failure to obtain growth in rats on a diet containing 25 per cent. of dried mangolds as the only source of vitamin A. Apparently this experiment has never been repeated. Certain results obtained by the present writers in a previous experiment on the influence of diet on the vitamin content of butter suggested the possibility that different varieties of mangolds might contain different quantities of vitamin A. Accordingly, four varieties of mangolds, Golden Globe, Intermediate, Long Red, and Golden Tankard, and one of swedes, were tested in quantities of 1 gm. fresh material per rat per day. This was about 10 per cent. of the diet and resulted in complete failure to promote growth (Fig. 1a). This work was carried out on mangolds in an immature condition in August.

In view of the belief of farmers that mangolds have higher feeding values in early spring than they have immediately after pulling in
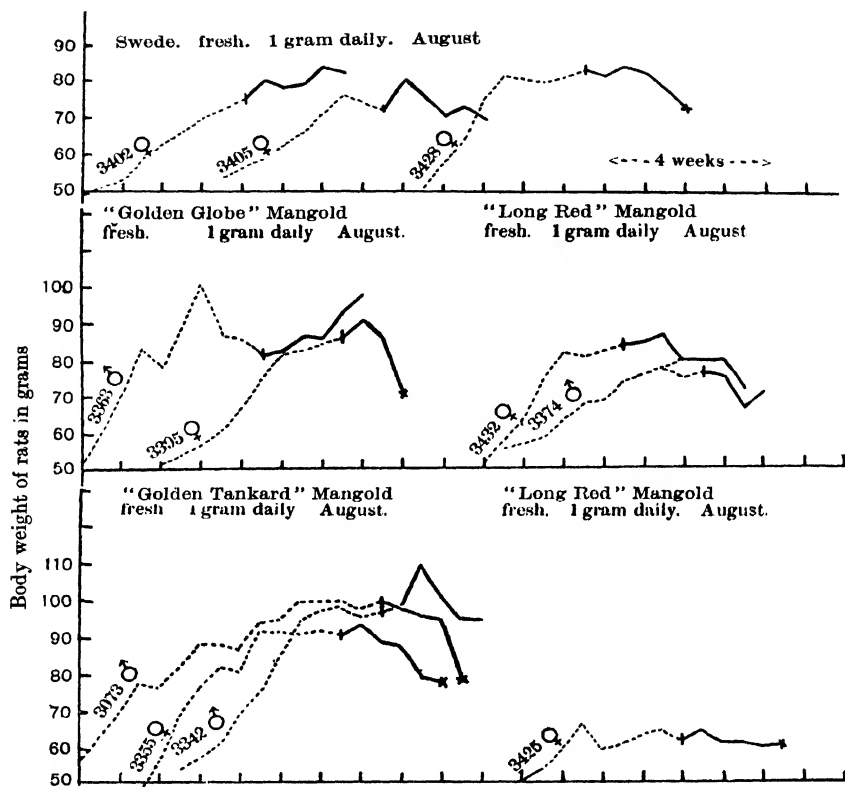
Fig. 1*a*.
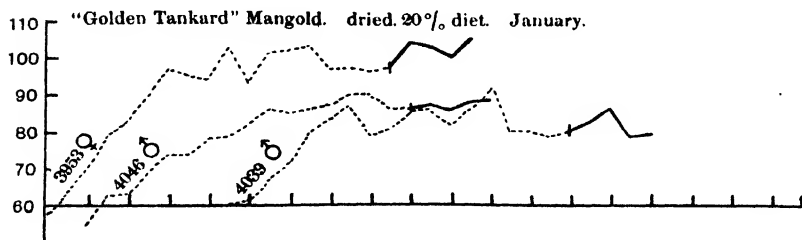
Fig. 1*b*.

early winter, and in order to test a larger dosage, the Golden Tankard mangolds were chopped up very coarsely in the early part of the year, partially dried at 37° C., in air and then more completely in vacuum at 60° C., and ground up to a fine powder. This powder was tested as 20 per cent. of the diet and yet failed to produce growth (Fig. 1*b*).

The fact that mangolds grow with their roots often half out of the ground and that the surface of the root exposed to the light is often greenish yellow, suggested the possibility of the formation of some vitamin A in this region (Coward, 1923). Three varieties of mangold were tested for this point in November (Fig. 2*a*). A thin layer (1 sq. inch by $\frac{1}{16}$ in.) of the upper surface was given to each rat per day. The Golden Tankard failed to give growth in this dosage, but the Intermediate Yellow Globe gave fairly good growth. The test was repeated the following March with samples of the same crops which had in the meantime been stored under the ordinary conditions of the farm. In this test the Golden Tankard was slightly active, the Yellow Globe inactive, and the Intermediate still active (Fig. 2*b*). Thus it would appear that mangolds, given whole to cattle may be a source of vitamin A, but the quantity obtained in this way must be very small indeed when the surface containing the vitamin A is compared with the whole bulk of the mangold which appears to be inactive.

(*b*) *Hay.* McCollum, Simmonds and Pitz (1916, 1) report the inadequacy of hay in growth-promoting factors, but it is uncertain from their data whether the fat-soluble or water-soluble vitamin was the limiting factor. Osborne and Mendel (1919) tested alfalfa, clover and timothy definitely for vitamin A. Their material "was growing vigorously when cut" and was dried at 50–60° C. The doses used were about 1 gm. of dried material daily per rat which appeared to Osborne and Mendel to be distinctly more than was necessary. Later (1920), they stated that a U.S.P. ether extract of leaves dried at 60° C. and given to rats in quantities equivalent to 1–2 gms. of the dried plant was sufficient to promote growth. Hart, Steenbock, Hoppert and Bethke (1922, 1) report a positive calcium balance in cows fed partly on alfalfa hay. Later (1922, 2), they report a negative calcium balance where timothy hay was used, and when this was replaced by alfalfa hay, a somewhat mitigated, though still negative, balance continued. On investigation it was found that the more beneficial hay had been "cured under caps," whereas the other had been "cured in the windrow with exposure to air and light for four days." Hart does not assume that the fat-soluble vitamin and the vitamin of plant tissue affecting calcium assimilation are identical, but rather

hesitatingly compares the low vitamin content of oat straw and its low power of increasing calcium assimilation with the much higher potency of the green straw found by Steenbock and Hart (1913). He also recalls his finding that "oat hay dried out of direct sunlight but in a fairly well-lighted attic appeared to retain the properties of fresh green oats" (1921).
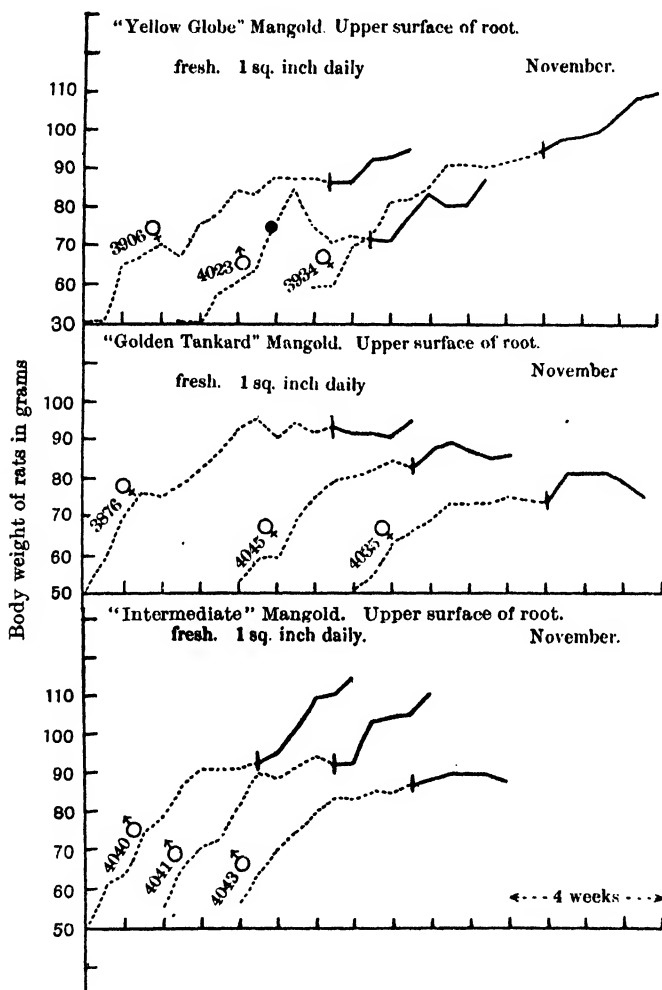
Fig. 2a. November.

We have confirmed the observations of these investigators that samples of hay may show very different vitamin A values. As will be described later the early stages of this year's experiment were much

disturbed by the fact that the sample of hay we were using was a good quality of fairly fresh meadow hay, consisting chiefly of perennial rye-grass and other bottom grasses with a small percentage of leguminous plants. As soon as we detected the influence that this component was having in maintaining the vitamin A value of the milk fat, it was changed
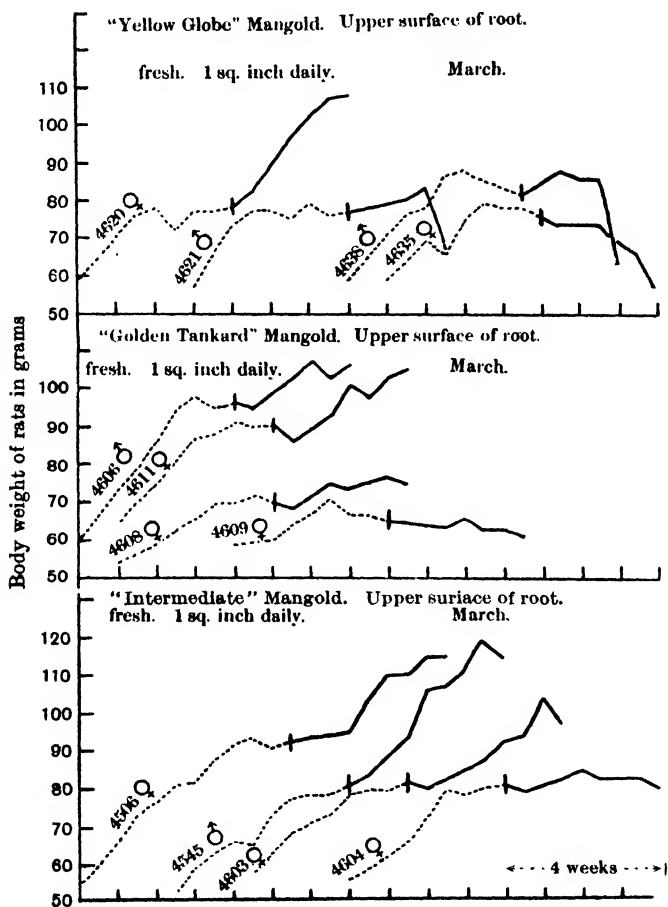
Fig. 2b. March.

for a seeds hay, much drier and more brown than the meadow hay used up to that time. Almost at once the vitamin A value of the butter fell. We think it very probable therefore, that good quality meadow hays which have been prepared under conditions which preserve to some extent the green colour of the constituent plants, may be most valuable

foodstuffs for cows during winter feeding and superior to the very dry brown hays. Curves are given in Fig. 3 showing the results of the feeding of meadow and seeds hays to rats.
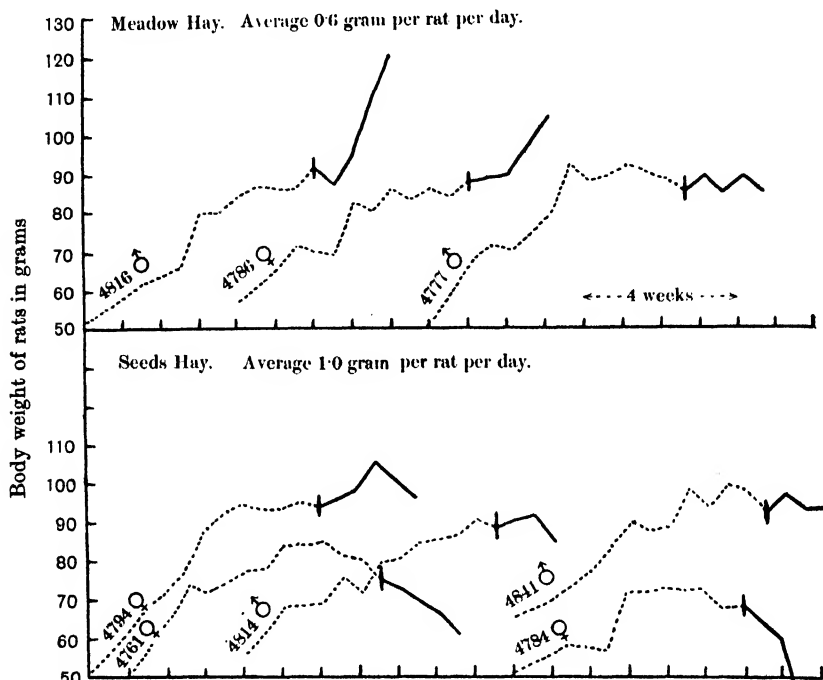
Fig 3.

(*c*) The sample of soya bean meal used in our experiment was not tested by feeding experiments on rats. It may have contained traces of vitamin A even though it had been fat-extracted, for Osborne and Mendel (1917) from experiments in which they used soya bean meal as 50 per cent. of a diet given to rats conclude that "the soya bean contains some of the fat-soluble vitamin." It is to be pointed out, however, that the meal used by these workers contained 14–20 per cent. fat. This finding was confirmed by Drummond and Zilva (1922), who found that soya bean oil, even when fed to rats in relatively large doses, gave only very slight growth. As the original beans also were poor in this factor it may be concluded that the soya bean cake used in the cow-feeding experiment contained only very small quantities of the vitamin.

(*d*) *Wheat.* McCollum, Simmonds and Pitz (1916, 2) have shown that wheat embryo contains small quantities of vitamin A; hence the addition

of 3 lbs. of whole wheat in the diet would supply small but definite quantities of this factor.

(e) *Maize gluten feed.* The method of preparation of maize gluten feed renders it unlikely that it contains more than traces of vitamin A, even if prepared from the yellow variety which is known to contain
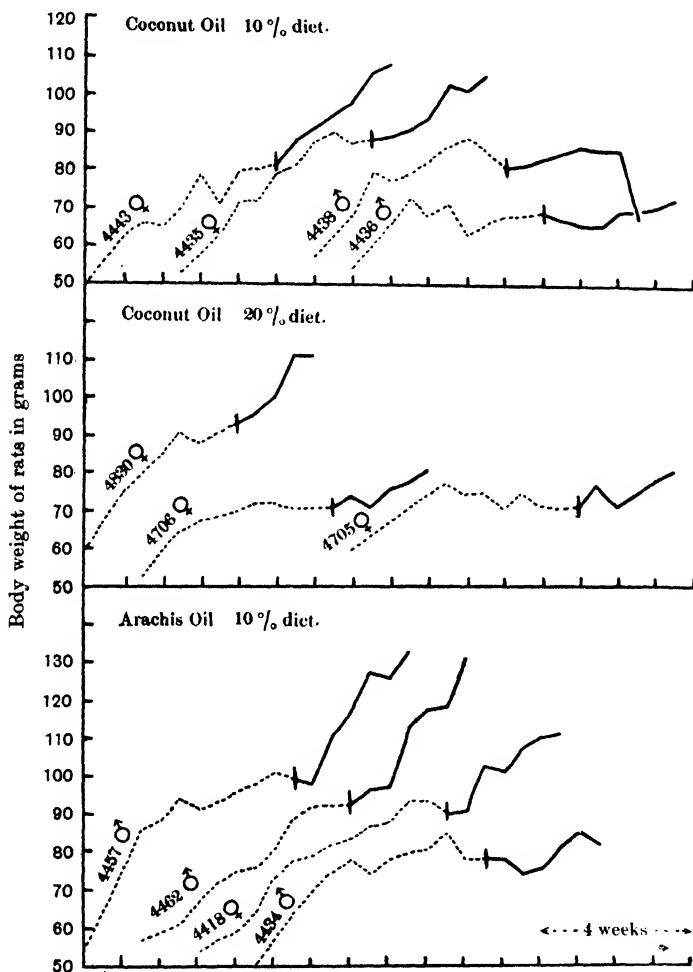
Fig. 4.

appreciable quantities. We have no information of the source of the sample used in our experiment.

(f) *The oils.* (i) *Cod liver oil.* The oil used was a clear yellow oil,

35—2

a daily dose of 5–7 mgms. of which restored growth to rats on a diet deficient in vitamin A.

(ii) *Arachis oil.* It was shown by Drummond and Zilva (1922) that arachis oil contains a small amount of the fat-soluble factor. Curves are given in Fig. 4 showing the effect of giving a diet containing 10 per cent. of this oil corresponding to a daily dose of about 1·0 gm. to rats, and the oil must have provided the cows with some vitamin A, although the amount given in the whole of period 2 (9·5 lbs. in all) was a very low percentage of the diet.

(iii) *Coconut oil.* Fig. 4 shows that coconut oil gave doubtful growth in rats even in a 10 per cent. diet (*i.e.* daily dose, 1 gm.), and the amount of vitamin A supplied to the animals in our experiment in this form must have been extremely small.

PROGRESS OF THE EXPERIMENT.

Throughout the experiment, until the final period of grass feeding, the cows were kept in stall, being only allowed out for a period of about 15 minutes every morning into a clean yard. Records of milk yield were made at both morning and evening milkings, and samples of both milks were submitted for determinations of specific gravity and fat, total solids being calculated. The food consumption of each cow was measured daily, and close observation of the general conditions of the animals was maintained.

The duration of the experiment can conveniently be divided into five main periods:

Period	Dates	Diet
1	12. xii. 22 to 26. ii. 23	Basal diet: concentrates roots *meadow* hay
2	27. ii. 23 to 14. iii. 23	Basal diet: as above and daily doses of coconut or arachis oil
3	15. iii. 23 to 16. iv. 23	Basal diet: concentrates roots *seeds* hay
4	17. iv. 23 to 17. v. 23	Basal diet plus cod liver oil
5	18. v. 23 to 11. vii. 23	Pasture. "Scarlet" received further cod liver oil for 17 days

Unfortunately in the early stages of Period 2 one of the cows—Fillpail—receiving coconut oil died suddenly. Efforts to determine the cause of death were unsuccessful; the other cow receiving coconut oil—Scarlet—lost her appetite towards the end of this period; she recovered rapidly after the end of the coconut oil period. The results from the butter fat from Fillpail have been omitted in this paper.

Method of Administering the Oils.

The oils were given in amounts beginning at 2 ozs. per day and rising to 8 ozs. as the cows became accustomed to them. During the oil periods, the total weights of oil given to each cow in lbs. was:

	Period 2 (27. ii. to 14. iii.)			Period 4 (17. iv. to 17. v.)		
	Lily	Lucy	Scarlet	Lily	Lucy	Scarlet
Arachis oil	9·5	9·5	—	—	—	—
Coconut oil	—	—	9·5	—	—	—
Cod liver oil	—	—	—	10·75	10·75	10·75

The oils were given at first at the morning feed only, but later when larger doses were being administered, at both morning and evening feeds. They were mixed with the concentrates and were readily taken by the cows.

The food consumption of the cows was satisfactory throughout the experiment. Such small variations were encountered in the amount of food eaten that the average figures for each period may be accepted as representing the daily intake.

Table II. *Average daily diet in lbs. during the various periods.*

Period	Date	Diet	Lily	Lucy	Scarlet	Remarks
1	12. xii. to 26. ii.	Mangolds	52·7	51·6	50·5	—
		Meadow hay	15·9	15·7	16·0	
		Concentrates	9·1	11·1	11·2	
2	27. ii. to 14. iii.	Mangolds	60	58	58	Lily and Lucy received
		Meadow hay	15	15	15	arachis oil, Scarlet
		Concentrates	9	12	8·4	ʼnut oil
3	15. iii. to 16. iv.	Mangolds	60	60	60	—
		Seeds hay	15	15	15	
		Concentrates	7·6	12	8·3	
4	17. i v. to 17. v.	Mangolds	60	60	60	All cows received cod
		Seeds hay	15	15	15	liver oil
		Concentrates	8	12	8	

In the final period (Period 5), all three cows were let out to pasture which was in very good condition. The administration of cod liver oil was discontinued except in the case of Scarlet who continued to receive further amounts.

Results.

(1) *Yield of milk and milk fat.* Daily records of these values were carefully taken, but for purposes of simplifying the charts by eliminating minor fluctuations, we give in Fig. 5 curves showing the average daily weight of milk and fat, and the fat percentage for each cow over periods of one week. In general it may be said that the curves given in Fig. 5

resemble the lactation curves of normal animals, but there is, in the case of all three cows, a definite fall in the percentage of fat during the period in which supplements of cod liver oil were given. This fall is promptly
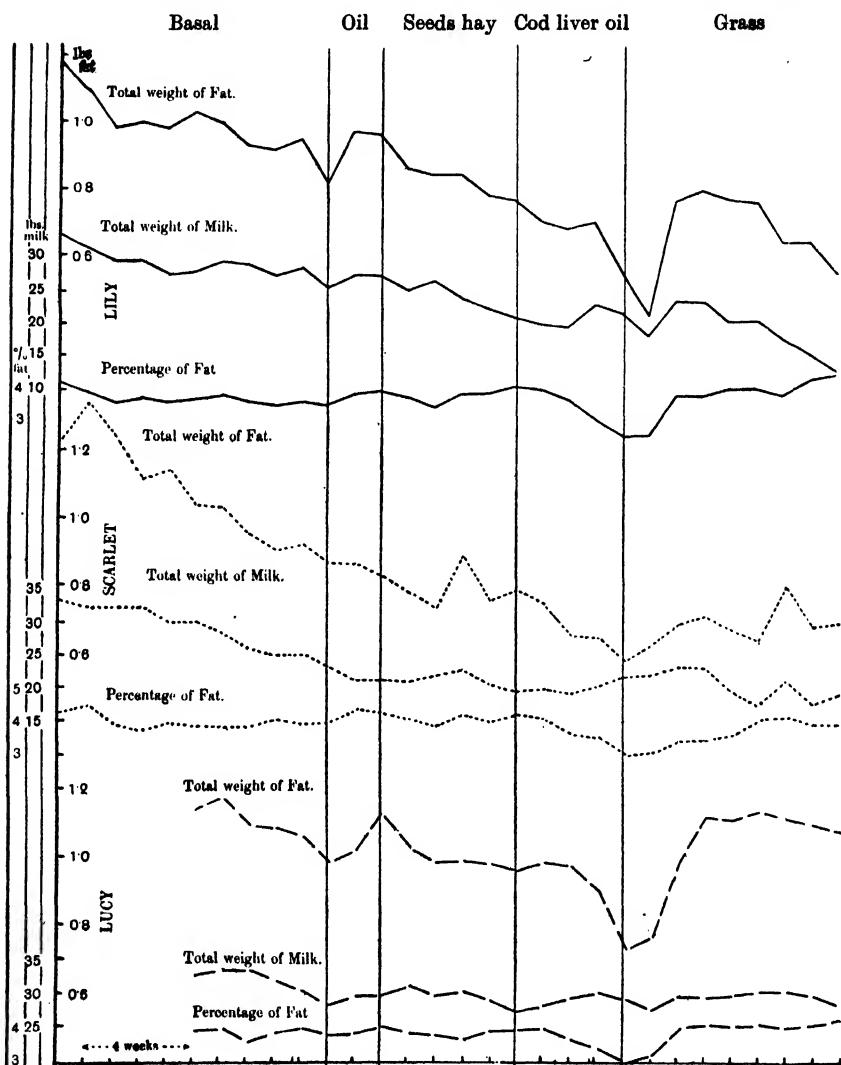
Fig. 5.

made good as soon as the cows were put out to grass in Period 5, even in the case of Scarlet who received cod liver oil for a time after she had been put out to pasture. We are not prepared at this stage of our enquiries

to give an opinion on the fall in the fat content of the milk during the cod liver oil period. It would appear to be of an order definitely greater than that which may occur during the later stages of lactation, and further experiments will be necessary in order to determine whether cod liver oil in relatively large doses can exert an inhibitive action on the amount of milk fat formed. No such effect was noticed in last year's experiment already reported in this Journal, but in those cases smaller doses of ½–4 ozs. were given. It will be recalled that in Hopkins' experiment on goats, the supplementing of a ration of mixed cereals, hay and mangolds, with green food, caused a bigger secretion of milk, but did not influence the proportion of total solids or fat (1920).

(2) *Quality of the milks and butters.* All the samples of milk from which the butters were made, were closely observed during churning. No sample of milk was found to possess a "fishy" flavour, but considerable abnormalities were noticed in certain samples during butter-making. This refers particularly to the butters yielded during Period 2, in which coconut and arachis oils were being administered to the cows. Butters of very low melting point were encountered during this, and in such cases, the churning presented difficulties. The prepared butters were entirely free from "fishy" flavour, even when the cows were receiving 8 ozs. (227 gms.) of cod liver oil daily. During the periods of oil feeding, however, certain of the butter samples tended to be a little oily, but this was chiefly when the animals were receiving the vegetable oils in Period 2.

The chemical examination of the butters was made in order to ascertain, how, under the carefully controlled conditions of this experiment, the composition was affected by the oil feeding. The results are forming the subject of a separate communication (in the press: to appear in *The Analyst*), but they may be summed up as follows:

(a) *Arachis and coconut oils.* No marked effect on the composition of the butter fat occurred during the period when these oils were given to the cows, but the melting points of two samples from the animals receiving arachis oil fell as low as 14·5 and 21° C., and the refractive indices showed a distinct rise.

(b) *Cod liver oil.* All the analytical constants showed abnormalities in the butters during the cod liver oil period, possibly because this oil was given for a considerably greater length of time. The iodine values rose to figures over 50, and the refractive indices showed a corresponding increase, reaching 1·4560. The saponification values fell to about 220, and the amount of water-soluble fatty acids decreased in proportion. The cod liver oil did not exert any marked effect on the melting points

of the butter-fats. It is interesting to note that these values did not return to their normal levels even after the cows had been out at grass for a period of 8 weeks.

(3) *Pigmentation of the butters.* The depths of colour of the various butter-fats were studied by comparing a tube of the melted fat with a set of arbitrary standards made from colourless liquid paraffin coloured with a fat-soluble yellow dye used for the artificial colouring of margarine.
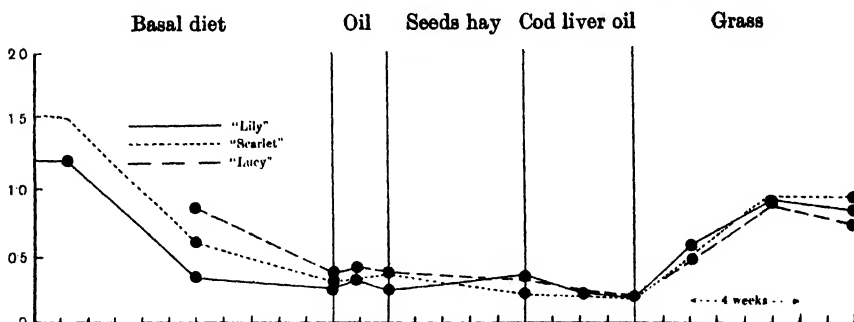
Fig. 6. Ordinates give arbitrary but comparative units of colour.
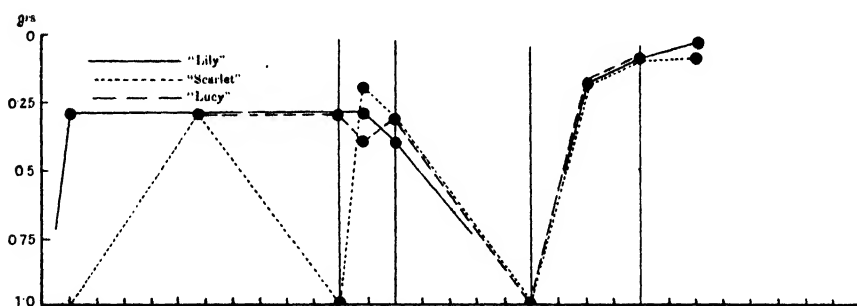
Fig. 7. Ordinates give the daily dosage of butter necessary to restore normal growth to rats on a diet deficient in vitamin A.

It is shown in Fig. 6 that the decrease in pigment was very gradual, and did not reach its lowest values until the beginning of the first oil period. It remained constant until the end of the cod liver oil period, during which time the butters were almost white. On turning the cows out to grass, the rate of recovery in pigment value corresponded very closely to its previous fall.

(4) *Vitamin content of the butters.* The vitamin A content of the butters was determined in the usual way by feeding experiments on

rats. The fluctuations in the vitamin value of the butters from the milk of each cow throughout the experiment are shown in Fig. 7. It will be seen that the activity of the butters showed no decrease on the basal diet even after 10 weeks, the daily dosage necessary to produce normal growth in rats being about 0·3 gm. From the work carried out in the previous experiment, it had been expected that the vitamin activity would have rapidly decreased. The reason for its failure to do so, was, as remarked earlier, found to be due to the relatively high vitamin content of the meadow hay, in the basal diet. On substituting for this meadow hay a brown seeds hay, the vitamin value of the butter decreased to such an extent that it required 1·0 gm. of it as a daily supplement to a vitamin-deficient diet to restore normal growth in rats. At this point, administration of cod liver oil was begun, and in three weeks the vitamin content of the butter rose rapidly, and the supplement which would cause resumption of growth in rats had become as low as 0·2 gm. Within a further fortnight, this value had decreased to 0·1 gm. The cause of the wide variations in Scarlet's butters, in the first two periods, has not been ascertained. Otherwise, the close parallel between the results of the tests is most striking, and relieves us of any anxiety as to the reliability of the results described last year, in which samples of butter from mixed milks had been used.

Discussion.

In all essential points the results of this experiment confirm those which we described in this Journal last year. It would appear definitely established now, that on a winter ration of concentrates, roots and hay, the vitamin A content of butter from the milk of cows in stall tends to fall. The composition of this winter feed, as Kennedy and Dutcher pointed out in 1922, may be such that the fall of vitamin A value in the milk is almost negligible. This is also seen in the first period of our experiment in which, by ingesting a liberal amount of fresh green meadow hay, the animals were able to maintain the vitamin concentration of their milks almost at summer level. When, however, a dry brown seeds hay was substituted for the superior green fodder, the characteristic fall in the nutritive value of the butter immediately occurred. This again emphasises the importance of controlling by rat-feeding experiments the vitamin value of all the components of a diet in an experiment of this type. Further, these results provide support for the opinion of Kennedy and Dutcher that a milk rich in vitamins is not necessarily correlated with access to pasturage.

There can be no doubt that the administration of cod liver oil in doses up to 4 ozs. daily will guard against a falling of the amount of vitamin A in the milk, and that larger doses will proportionately raise the vitamin value of that product. In view of our results, however, it would be wise not to recommend the higher dosages of this oil in the winter feeding of cows until it has been ascertained whether or not the decrease in the percentage of fat in the milk during the cod liver oil period (4) is attributable to the oil itself or to other factors.

SUMMARY.

(1) Further experiments are described, the results of which support the relationship between the presence of vitamin A in the diet of the cow and its presence in milk fat.

(2) The typical winter ration of concentrates, roots and hay may be adequate to maintain the vitamin A value of the milk fat for considerable periods provided that at least one of the components supplies adequate amounts of that dietary principle. Well cured green meadow hays are in this respect greatly superior to dry brown seeds hays.

(3) The addition of cod liver oil to a winter ration deficient in vitamin A will induce a sharp rise in the vitamin A value of the milk fat of cows. No such effect is seen when oils deficient in this dietary factor (coconut oil, arachis oil) are given.

(4) The administration of cod liver oil in doses from 1 to 8 ozs. daily to milking cows caused no "fishy" taint in the milk or butter-fat.

(5) The administration of cod liver oil caused no appreciable change in the yield of milk but the higher doses appeared to cause a noticeable drop in the percentage of fat. Further experiments are required to throw more light on this observation.

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A NEW METHOD FOR THE DETERMINATION OF AMMONIACAL NITROGEN IN SOILS.

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THE aeration method of Matthews [1] is generally recommended in this country for the determination of ammoniacal nitrogen in soils. Whilst that method is satisfactory in laboratories possessing a good water supply, it is somewhat precarious in laboratories where, as at Bangor, the water pressure is badly maintained. In our own experience of the aeration method, a considerable proportion of determinations are spoilt owing to changes in water pressure. In the case of fine-textured soils, failure in the aspiration of the air current leads to blocking up of the tube and aeration cannot again be started.

An attempt was, accordingly, made to devise a method suitable for routine work under conditions where a satisfactory supply of water is not available. It occurred to the authors that any ammonium present in the soil should be in a zeolitic or exchangeable state analogous to the exchangeable bases estimated by the Hissink leaching method [2]. The work of Gedroiz [3] and of Hissink has rendered probable the existence in soils of a definite content of exchangeable bases. If there is also a definite content of exchangeable ammonium it should be quantitatively displaced by the leaching process used in the Hissink method for the determination of exchangeable bases. In other words, the Hissink method should be applicable to ammonium as well as to calcium, magnesium, potassium and sodium.

This has been investigated at Bangor during the past year. A number of soils of different character have been submitted to leaching by the Hissink method, using sodium chloride solution, and ammonium has been determined in the filtrates obtained. The results have been compared with those for the same soils by the aeration method.

EXPERIMENTAL.

In the earlier experiments, sodium hydroxide was used to liberate the ammonia from the leachings. The results shown below for a soil from the college farm give an idea of the figures obtained.

Aeration method gave 54 parts per million ammoniacal N.

Leaching method, using normal sodium chloride solution, gave: in 1st half litre, 68·3 parts per million N; in 2nd half litre, 11·2 parts per million N; in 3rd half litre, 3·3 parts per million N.

These are typical of a large number of experiments in which it was found that the first half litre gave higher results than the aeration method and that appreciable, though smaller, quantities were obtained in subsequent washings. This suggested that the treatment with sodium hydroxide liberated small quantities of non-ammoniacal compounds decomposable by sodium hydroxide. It was therefore decided, in all subsequent experiments, to use magnesium oxide for the liberation of ammonia. The figures with sodium hydroxide may, however, be of some significance as representing a certain level of nitrogen availability.

Method Adopted.

It will probably be simpler, before discussing the effect of certain variations in working details, to give an account of the method as finally adopted.

Twenty-five grams of soil, from which material coarser than 3 mm. has been removed, are weighed out into a 400 c.c. beaker. About 100 c.c. of cold normal sodium chloride solution are then added. The contents of the beaker are stirred at intervals for a few minutes until the soil is thoroughly moistened, and allowed to stand for about half an hour. The clear supernatant liquid is poured on to a large (18·5 cm.) filter from which the filtrate is collected in a litre Pyrex flask. The soil is washed again with sodium chloride by decantation and the contents of the beaker finally transferred to the filter. Washing with sodium chloride is carried on until half a litre of filtrate has been collected. It will be noticed that the leaching is carried out entirely in the cold. The filtrate is then directly distilled with excess of magnesium oxide into a measured quantity (10 to 15 c.c.) of $N/50$ sulphuric acid. After distilling for about half an hour during which about 150 c.c. of distillate have been collected, the excess of acid is titrated with $N/50$ sodium hydroxide. Methyl red may be used for indicator as in the Matthews method. We have, however, found that B. D. H. Universal Indicator gives a much sharper end point. The titration should be carried to a distinct blue. Using 25 grams of soil, titration to 0·05 c.c. gives an accuracy of ·56 part per million of nitrogen.

The reagents used were of analytical quality and blank estimations were carried out from time to time as a check.

In some cases 250 c.c. of 15 per cent. sodium chloride were used

instead of 500 c.c. of the normal solution. In the case of soils containing high proportions, say over 50 parts per million of ammoniacal nitrogen, a second half litre of leachings should be collected if normal sodium chloride is used, or 500 c.c. of 15 per cent. solution may be used instead.

As a rule six determinations are started at once. With two distillation apparatuses, the six can be completed within 24 hours.

EFFECT OF VARIATIONS IN WORKING CONDITIONS.

We shall now proceed to give an account of the effect of certain variations in the working conditions.

(a) *Successive Leachings.* The following table shows the results of determinations of ammoniacal nitrogen made on successive leachings. Normal sodium chloride solution was used in all cases except in the case of the Cedar Hill soil, where 10 per cent. solution was used.

Table I. Ammoniacal nitrogen in successive leachings.
(Parts per million.)

Soil	Aeration method	Leaching method	
		1st 500 c.c.	2nd 500 c.c.
A 77 A ...	31·7	{31·7	4·4
		31·7	4·4
Coll. Garden ...	10·1	{10·1	1·1
		10·1	2·2
A 72 A ...	28·3	{29·4	3·3
		28·3	4·4
Farm Soil ...	35·0	{38·5	1·1
		39·1	1·1
Cedar Hill ...	159·0	154·0	6·8
Ditto. 67 p.p.m. N as NH₄Cl added	229·0	223·0	5·6

The recovery is thus practically complete in the first half litre of leachings, and for ordinary purposes it is probably sufficient to use this amount. With very high proportions of ammoniacal nitrogen it may be necessary to take a second half litre of leachings or to use more concentrated sodium chloride solution.

(b) *Speed of Reaction.* According to Hissink, base exchange is practically instantaneous. If this be the case, prolonged reaction of the soil with the leaching solution should be unnecessary. The following experiments relate to this point. A garden soil, giving 124·9 parts per million ammoniacal nitrogen by the aeration method was leached with 500 c.c. normal and 15 per cent. sodium chloride solution respectively. Varying times of contact were used in each case and the results are set out in the following table.

Table II. Ammoniacal N in parts per million. Varying times of reaction.

Time	Normal NaCl	15 % NaCl
15 mins.	116·5	123·2
2 hrs.	115·4	126·2
6 hrs.	118·2	122·1
24 hrs.	115·4	123·8

It will be seen that the reaction, as suggested by Hissink for other adsorbed bases, is practically instantaneous and the results for four different times can only be regarded as replicates. There is thus no need for long standing and after the soil has been thoroughly moistened with the leaching solution, a period of half an hour is probably ample, since the washing also occupies time in addition. The above experiment also shows that with high proportions of ammoniacal nitrogen, half a litre of normal solution is insufficient, even with prolonged contact. With 15 per cent. solution, the agreement with the aeration method is sufficiently good.

(c) *Strength of Sodium Chloride Solution.* Whilst the displacement of ammonium from soil can be effected by the use of relatively dilute solutions, the reaction might be expected to require bigger volumes of leachings for completion. The following experiment was carried out to ascertain if a solution more dilute than normal could be used with advantage.

Soil	500 c.c. N NaCl	500 c.c. $N/2$ NaCl
27 A	11·2 p.p.m.	6·7 p.p.m.
C 31	20·2 ,,	17·4 ,,

It would appear that the use of seminormal sodium chloride offers no advantage as it would be necessary to use a larger volume of leachings. With regard to the possibility of using a smaller volume of a more concentrated solution, the figures in Table IV may be considered. From these it appears that for ordinary soils, 250 c.c. of 15 per cent. sodium chloride gives practically the same result as 500 c.c. of normal solution. The point will, however, be discussed later when comparing the results with those obtained by the aeration method.

(d) *Recovery of added Ammonium Salts.* It was thought that an estimation of the recovery of added ammonium salts would serve as a check on the method. Known quantities of a standard $N/50$ solution of ammonium chloride were used to moisten certain soils. Ammoniacal nitrogen determinations were then carried out by the leaching method. The following table gives the results obtained, with the percentage recovery.

Table III. Recovery of added Ammonium Salts.

Soil	Ammoniacal N	N added p.p.m.	Ammoniacal N in treated soil	Recovery %
F 4	13·4	112·0	124·3	99·0
C.H.	154·1	67·0	223·0	102·8
Tarouba	11·4	61·0	72·8	100·8
Garden	3·4	53·6	57·3	100·5

It is seen that the recovery is practically quantitative. That the recovery is greater than 100 % in the case of soil C.H. is probably due to the fact that the figure for ammoniacal nitrogen in soil alone is on the low side.

AGREEMENT WITH AERATION METHOD.

From some of the figures already given it has been seen that there is generally a good agreement between results by the new method and results by the aeration method. The results obtained are collected together in Table IV which shows the comparison between the two methods.

Table IV. Comparison of the Leaching and the Aeration Methods.

Soil	Ammoniacal N, p.p.m. Leaching method		Aeration method
	500 c.c. N NaCl	250 c.c. 15 % NaCl	
27 A	—	11·2	11·2
C 31	20·2	21·8 (500 c.c.)	22·9
C 23	12·9	12·3	12·9
D 60	27·4	24·0	27·4
F 1	11·2	12·3	11·8
F 7	—	15·1	15·1
F 8	3·7	5·3	4·0
F 21	—	7·8	7·2
F 22	6·2	5·8	6·2
Garden	3·4	—	3·4
47 A	10·0	9·9	9·1
D 2	8·5	9·4	10·1
		10·1 (500 c.c.)	
48 A	17·9	16·6	17·3
		17·9 (500 c.c.)	
D 6	7·8	6·0	6·7
		6·7 (500 c.c.)	
D 1	22·4	20·7	21·6
F 17	21·3	21·3	19·6
Garden	10·1	—	10·1
A 72	28·3	—	28·3
Aber	38·5	—	35·0
A 77	31·7	—	31·7
Cedar Hill	136·5	142·9	159·1
		154·1 (500 c.c.)	
Rich garden soil	116·5	123·2 (500 c.c.)	124·9

If the last two soils, containing abnormally high proportions of ammoniacal nitrogen be omitted, and all the comparisons between the aeration method and the leaching method using 500 c.c. N NaCl be

averaged, then, putting the aeration results at 100·0, the results with the leaching method work out also at 100·0. Similarly, comparing the results with 250 c.c. of 15 per cent. NaCl with the aeration method, the leaching method works out at 98·6, a satisfactory agreement, but indicating a slightly smaller recovery. With the two soils containing high proportions of ammoniacal nitrogen, it will be noticed that the agreement is not good either with 500 c.c. N NaCl or with 250 c.c. of 15 per cent. NaCl, but that a better agreement was obtained with 500 c.c. of 15 per cent. solution. In cases where a high proportion of ammoniacal nitrogen is found in the first half litre of leachings, a second half litre should be collected if normal solution has been used, or if a high figure be suspected, 15 per cent. solution may be used at the outset and half a litre of leachings collected.

The remarkable agreement between the results obtained by two methods is evidence that we are dealing with a definite quantity and not, as in the case of "available" phosphoric acid in soils, a quantity conditioned closely by methods of working. Ammoniacal nitrogen, although, as will be shown in later work, subject to great variations in the same soil, is at a given time a definite amount and does not depend on the details of a conventional method. It is a considerable advantage to be exempt from the necessity of rigidly prescribing working conditions as is done, for example, in the estimation of "available" phosphoric acid. In the present instance some latitude in working details is permissible so long as the displacement of ammonium is complete. This seems to be effected in ordinary soils by the use of 500 c.c. of normal, or 250 c.c. of 15 per cent. sodium chloride solution. Where larger amounts of ammoniacal nitrogen are present, 500 c.c. of the stronger solution may be required.

Whether it is possible to distinguish between exchangeable and acid soluble ammonium as is done by Gedroiz and Hissink in the case of calcium and similar metals, we cannot be certain. For example, the method of determination of acid soluble calcium follows from the definition. In the case of ammonium, however, treatment with acid might be expected to lead to the hydrolysis of some of the simpler nitrogenous compounds present, with consequent production of ammonium salts. Further, ammonium is not an element found in natural silicate minerals. It is probably not unreasonable to assume that all the ammonium actually present in the soil at the time of estimation is actually recovered in the leachings.

The leaching method has the advantage over the aeration method that it does not require expensive apparatus and may be employed where

a high pressure water supply is not available. Owing to the simplicity of the manipulation it should be more suitable for routine purposes than the aeration method. Six determinations per day may easily be completed. The aeration method may possibly be more rapid where the water supply is sufficiently good to enable a battery of six tubes to be run in series. This condition cannot always be relied upon in provincial laboratories.

SUMMARY.

A method for the determination of ammoniacal nitrogen in soils is described. It is an extension of the Hissink method for exchangeable bases to ammonium present in the soil. The working details are similar to the Hissink method, except that the leaching process is entirely carried out in the cold. The ammonia is distilled off with magnesium oxide.

Using normal sodium chloride as a leaching solution and collecting half a litre of leachings for distillation with magnesia, results were obtained which showed excellent agreement with those by the aeration method. Similar results were obtained using 250 c.c. of 15 per cent. sodium chloride solution, but the results are on an average about 1·6 per cent. lower. With high proportions of ammoniacal nitrogen a second half litre of normal sodium chloride leachings should be taken, or half a litre of 15 per cent. solution used.

The leaching method is economical of apparatus and water supply. It is therefore suitable for small laboratories with limited resources. It is fairly rapid: six estimations can be completed in a day.

The close agreement of the new method with the aeration method suggests that the amount of ammoniacal nitrogen in the soil at any given time is not an arbitrary quantity depending on conditions of estimation, but a definite amount. It is improbable that any ammonium compounds exist in the soil apart from those which take part in base exchange.

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INFLUENCE OF MICROORGANISMS UPON THE CARBON-NITROGEN RATIO IN THE SOIL[1].

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VARIOUS investigations on the decomposition of organic matter in the soil have brought out the fact that there exists a more or less constant ratio between the carbon and nitrogen content of the soil, whatever the ratio between these elements in the organic matter originally added to the soil. This ratio varies from 8 : 1 to 12 : 1, *i.e.* for every 8 to 12 parts of carbon, there exists in the soil one part of nitrogen; the average ratio is about 10 to 1. Brown and O'Neal (1923), for example, found that the ratio of the carbon to the nitrogen in a Carrington loam is 12 : 1 to 13 : 1, while, in the case of a Tama silt loam, the ratio may go down to 10 : 1. According to Sievers (1923), the ratio of carbon to nitrogen in the soil is about 11·6 : 1. Russell (1923) stated that, although there is about 40 times as much carbon as nitrogen in the original plant residues, the ratio will drop down to 10 to 1, before these residues have been very long in the soil. This ratio seemed to be in a stable position, for which no explanation could be suggested. Fraps (1922) found the ratio of carbon to nitrogen in the surface soil to be 9·2 : 1 and in the subsoil 8·3 : 1; he suggested, therefore, to judge the percentage of organic carbon in the soil from the percentage of nitrogen present.

No attempt has ever been made, so far as the writer is aware, to establish the reason for this particular ratio between these two important constituent elements in the soil. An attempt will be made in this article to explain how this carbon-nitrogen ratio in normal cultivated soils may be, to a large extent, a result of the activities of the microorganisms inhabiting the soil.

After an organic substance is added to the soil, it is acted upon by various groups of microorganisms, including the fungi, actinomycetes and bacteria and possibly also the protozoa. A part of the organic matter is completely decomposed, with the formation of CO_2, H_2O, NH_3, H_2S, etc.; another part is reassimilated by the organisms and

synthesised into protoplasm; a part is left undecomposed, being more resistant to the action of microorganisms; a part is left in the form of intermediate products, due either to the greater resistance of these to the action of the microorganisms in general and to certain specific groups in particular, or to the fact that products are formed hindering the further development of the organisms.

This is true of substances of mixed composition, such as straw, or other natural products. However, in the case of purified organic materials, even the most complex, like celluloses, starches and proteins, it can be readily demonstrated that none of the original materials are left undecomposed, after a short period of time, if conditions for their decomposition are favourable. In this case, all the resulting products can be accounted for by: (1) the final waste products, such as CO_2, NH_3, H_2O, etc.; (2) the synthesised protoplasm of the microbial cell and (3) by certain intermediary products, which are not decomposed completely for one reason or another. Some of these intermediate products, such as citric, oxalic and fumaric acids are formed by fungi; lactic, acetic and butyric acids are formed by bacteria; these acids will be decomposed further as soon as a basic substance is added, sufficient to neutralise these acids.

The quantities of carbon and nitrogen reassimilated and resynthesised into microbial protoplasm depend upon the nature of the organism, the amount of energy made available in the process of decomposition of the organic matter, and the amount of available nutrients, especially nitrogen (to a less extent phosphorus), necessary for the synthesis of the protoplasm. The fungi will reassimilate between 20 to 60 per cent. or, on the average, 30 to 40 per cent. of the carbon of the substratum that has been decomposed; the bacteria will reassimilate between 1 and 30 per cent., or on the average, about 5 to 10 per cent. of the carbon used as a source of energy. The fact that the fungi will reassimilate a much greater amount of the carbon of the nutrient that they have decomposed than the bacteria, has been established by a number of investigators, as shown by Kruse (1910).

The nitrogen content of the water-free fungus protoplasm is 3 to 8 per cent., depending on the composition of the medium, being 3·5 to 6 per cent. in carbohydrate rich, protein poor media and about 5 to 8 per cent. in carbohydrate poor, protein rich media; on the average, we may assume that the fungus protoplasm contains 5 per cent. of nitrogen and about 45 to 50 per cent. of carbon. The water-free cells of bacteria contain about 10 to 12 per cent. of nitrogen (also depending to

some extent upon the composition of the substratum) and about 45 to 50 per cent. carbon. The actinomycetes stand midway between the fungi and bacteria, assimilating about 15 to 30 per cent. of the carbon of the substratum and containing about 7 to 10 per cent. nitrogen.

The extent of decomposition of carbohydrates by microorganisms is found to depend on the nature of the organism and the amount of available nitrogen. For every unit of carbon decomposed as a source of energy, a certain amount of nitrogen is assimilated, independent of the fact as to whether the latter is present in the form of proteins, simple protein degradation products or inorganic salts; only the energy balance will be different. In this respect the fungi differ distinctly from the bacteria; the former assimilate a great deal of the carbon that is decomposed and, although their nitrogen requirement is less than that of bacteria, the total amount of nitrogen assimilated will be found to be much greater, due to the comparatively much smaller synthesis of protoplasm by the bacteria.

To take a hypothetic example: if 100 units of organic matter (assuming it is nitrogen-free) are decomposed by fungi (the carbon serving both for energy and structural purposes), as much as 40 to 50 parts of its carbon may be reassimilated by the organisms; the C : N ratio of the fungus protoplasm is about 9 : 1. Assuming that the carbon content of the organic matter added to the soil is the same as that of the fungus mycelium, we will find that fungi will require, for every 100 units of organic matter decomposed, 1·5 to 2·5 units of nitrogen: 100 units of organic matter decomposed allow the synthesis of 30 to 50 units of fungus mycelium containing 5 per cent. of nitrogen.

The amount of nitrogen required may even be higher, since certain fungi may contain 6 to 7 per cent. nitrogen. For the complete decomposition of every 100 pounds of cellulose, therefore, about 2·5 pounds of available nitrogen are required; for the complete decomposition of 100 pounds of straw which contains about 0·5 per cent. nitrogen, 1·5 to 2·0 pounds of additional nitrogen will be required, if fungi are the only organisms concerned in the process; for the decomposition of every 100 pounds of alfalfa, containing 3 per cent. nitrogen, no additional nitrogen is required, a small amount of nitrogen may even be liberated in the form of ammonia. In the case of organic substances containing more than 2·5 per cent. of nitrogen, the excess of the latter will be left as a waste product, in the form of ammonia. The greater the nitrogen content (not the total amount of nitrogen, but the percentage) of the particular organic substances added to the soil, the greater will be the

amount of nitrogen becoming available. In the case of dried blood containing, for example, 12 per cent. nitrogen, as much as 75 to 80 per cent. of the nitrogen may, in course of time, become available as ammonia; this is then transformed to nitrates. We must allow, however, for two phenomena: (1) a wide carbon-nitrogen ratio of the soil organic matter, in which case some of the nitrogen made available from the decomposition of the dried blood will be again assimilated by organisms capable of utilising the carbon compounds of the soil as sources of energy; (2) not all the dried blood is completely decomposed, some of it may be left in the form of more or less resistant intermediary products.

It is important to emphasise, in this connection, that the mechanism of decomposition of organic substances and especially the amount of ammonia liberated depends largely upon the amount and nature of the available energy in the particular organic matter or in the soil. This points to the absolute lack of justification in making comparisons of the rapidity of decomposition of different nitrogenous organic substances containing different amounts of nitrogen, using ammonia as an index of availability; different quantities of organic matter are usually employed of a different carbon-nitrogen ratio; when the same amounts of nitrogen are added, different amounts of carbon, and, therefore, of available energy, will be introduced, assuming that the carbon is equally available in all cases. More ammonia will be obtained from the decomposition of dried blood than from the decomposition of cotton-seed meal, not because the first is more available, but because the second contains half as much nitrogen, and, therefore, when the same amounts of nitrogen are used, twice as much carbon will be used with the second; the organisms using the latter as a source of energy will be able to store away twice as much nitrogen. Kelley (1915), for example, found that about the same amount of ammonia was formed from such different substances as casein, soy bean cake meal, cotton-seed meal and linseed meal, when enough starch was added, so that the material added to the soil contained the same amounts of nitrogenous and non-nitrogenous matter.

The bacteria will assimilate much less nitrogen than the fungi, although their cells contain a larger amount of nitrogen than the fungus mycelium, since the growth of the latter is much more abundant. In the case of various complex organic substances, many bacteria will either not decompose them at all or leave a large part of the decomposed material in the form of intermediary products. But even, for the same amount of energy consumed, bacteria will assimilate much less nitrogen than fungi, and, will therefore leave a great deal more nitrogen as a

waste product either in the form of ammonia or as other protein degradation products. If an organic substance of a low nitrogen content, like alfalfa, is acted upon by fungi, on the one hand, and bacteria, on the other, the former will either not liberate any ammonia at all or only small amounts of it, while the latter may liberate appreciable quantities of ammonia. This can easily be seen from the following table, where results on the formation of ammonia by fungi, actinomycetes and bacteria from soil alone and from alfalfa meal added to the soil are given.

Table I. Formation of ammonia from 100 gm. of sterilised soil and from 2 per cent. of alfalfa in 100 gm. of sterilised soil by different microorganisms.

(Incubation, 18 days at 25–28° C.)

Organism	Sterilised soil NH_3–N mgm.	2 % alfalfa in sterilised soil NH_3–N mgm.
Control	1·87	4·90
Trichoderma sp. ...	3·02	0·58
Penicillium sp. ...	2·89	0·86
Fusarium sp. ...	2·59	1·87
Act. californicus ...	3·17	5·47
Act. viridochromogenus	5·37	6·50
Bac. cereus	2·59	5·18
Bact. fluorescens ...	2·30	5·33

These two factors, namely (1) the nature of the carbon and nitrogen content of the soil and of the organic matter added to the soil, and (2) the activities of fungi and bacteria in the soil, will help to explain a great many phenomena that could not be explained up to the present time. Of these, we need point out only a few.

When organic matter is added to the soil, either the formation of ammonia followed by nitrate accumulation or nitrogen starvation will set in, or both will be about balanced. This will depend on the carbon and nitrogen content of the organic matter. If the nitrogen content of the organic matter is more than 2 or 2·5 per cent., no nitrogen starvation can set in, but ammonia will be liberated. If the nitrogen content of the organic matter is about 2 to 2·5 per cent., a temporary nitrogen starvation may set in lasting but a few days, followed by the liberation of ammonia. If the nitrogen content of the organic matter is about 1 per cent. or less, a lasting nitrogen starvation may set in, which may be corrected by the addition of inorganic nitrogenous fertilisers.

This has been definitely demonstrated by Rahn (1919), who found that in the presence of 0·5 to 1·0 per cent. of straw, the available nitrogen in the soil is rapidly used up and a nitrogen deficiency ensues. When

available nitrogen is added, the straw is rapidly decomposed and the soil becomes normal again, with a constant carbon-nitrogen ratio.

This will help to explain the results of Lyon, Bizzell and Wilson (1923), who added 0·6 gm. of nitrogen, in the form of roots of different plants, to 28 pounds of soil, incubated the soils for 3 months and leached out the soluble nitrogen (nitrates). The amounts of nitrogen recovered were proportional to the percentages of nitrogen in the substances added, as shown below:

Material used	Per cent. nitrogen	Weight of roots added gm.	Nitrogen in leachings mgm.
Control soil	—	—	946·6
Oats	0·45	133·3	207·3
Timothy	0·62	96·8	398·4
Maize	0·79	75·9	510·6
Clover	1·71	35·1	924·4
Dried blood	10·71	5·6	1751·1

There was about the same amount of nitrate formed in the control soil and in the soil to which clover was added, showing that about 2 per cent. of nitrogen was sufficient to allow the microorganisms to utilise all the available energy in the fresh organic matter. The greater the amount of organic matter added, the greater is the amount of available energy and, therefore, the greater is the amount of nitrogen that will be reassimilated by the microorganisms; this, combined with the lower nitrogen content, accounted fully for the decrease in the nitrate content of the leachings.

That fungi and actinomycetes, which synthesise an extensive mycelium, are probably more concerned in the decomposition of straw and other organic residues in the soil than the bacteria, which synthesise a correspondingly smaller amount of protoplasm, of a narrower carbon-nitrogen ratio, is further brought out by the following observation. When a large amount of straw is added to the soil, nitrogen starvation sets in, due to the above considerations. However, when the soil is treated with carbon bisulphide (Hiltner, 1908), during or soon after the addition of the straw, the phenomenon of nitrogen starvation is not apparent. The disinfectant destroys the soil fungi (Waksman and Starkey, 1923) and greatly reduces the number of actinomycetes, while the bacteria, although at first reduced in numbers, are greatly stimulated, after the disinfectant has evaporated. The above phenomenon may be due largely to the lower nitrogen requirement of the bacteria and also to the greater liberation of available nitrogen, as a result of the numerous processes following partial sterilisation of soil.

The above considerations explain why the carbon-nitrogen ratio

of the soil is more or less constant. It could not be otherwise: were the carbon content too large in relation to the nitrogen, the soil would not be in a condition to support plant growth, as long as this excess lasted; the microorganisms using the carbon as a source of energy would assimilate every trace of available nitrogen that would otherwise be made available for the growth of higher plants. Were the nitrogen content too large, ammonia would be rapidly liberated and then transformed to nitrates and either leached out or assimilated by higher plants. In the case of a soil with a constant carbon-nitrogen ratio of about 10 to 1, the activities of the microorganisms are in a condition of a more or less unstable microbiological equilibrium; under these conditions, only small amounts of energy are utilised, resulting in the liberation of corresponding amounts of ammonia, which are later transformed into nitrates. The amount of ammonia liberated in a soil under normal conditions depends on the amount and kind of organic matter in the soil as well as on the environmental conditions.

Any change in the physical, chemical and microbiological condition will result in a change in the unstable equilibrium, accompanied by an increase in the amount of organic matter decomposed, as indicated by the evolution of carbon dioxide, and, therefore, also by an increase in the amount of ammonia liberated. Among the phenomena which bring about such changes, we may mention the neutralisation of an acid soil with CaO or $CaCO_3$, the treatment of the soil with volatile antiseptics, steaming of soil or heating it to a temperature less than $98°$ C., drying followed by moistening, etc.

The fact that these treatments tend to destroy the fungi to a greater extent than the bacteria, the fact that the latter become active much more rapidly than the former after the treatment and, finally, the fact that the bacteria assimilate much less nitrogen than the fungi per unit of energy utilised or carbon transformed, all tend in the same direction, namely a greater liberation of ammonia than would have taken place in untreated soil.

Before we can understand the processes of decomposition of organic matter in the soil, resulting in the liberation of the available nitrogen, we must have a clear conception of the chemical composition of the organic matter, types of microorganisms acting upon it, nature of the chemical transformation brought about, as well as the environmental conditions under which these processes take place.

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A CHEMICAL STUDY OF THE DEVELOPMENT OF THE WHEAT GRAIN.

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(With One Text-figure.)

SUMMARY OF CONTENTS.

INTRODUCTION.

THE connection which has been shown to exist between the strength of wheat flour and the chemical individuality of the glutenine fraction of the wheat protein (Woodman, 1922)[1] led the writers to institute an enquiry into the manner in which the individual wheat proteins are developed and stored during the progress of the grain from the early stages after flowering to ripeness. The immediate object of the investigation was to secure information in regard to the stages at which the different proteins made their appearance in the grain and to determine

at what point the character of the grain contents was such as to enable a tenacious gluten to be obtained by grinding up the kernels with successive quantities of dilute NaCl solution. It was also intended to follow the rate of alteration of the amounts of the several proteins during the growth of the grain and to attempt to elucidate as far as possible the relationships which exist between the simple and complex forms of nitrogen at the various stages.

Numerous investigations have been made into the distribution of the nitrogen of wheat flour and the ripe wheat kernel, but comparatively little is known concerning the partition of the nitrogen during the immature stages and the alterations which occur as the seed ripens. Brenchley and Hall [2], in their extremely valuable work on the development of the wheat grain, contented themselves, in respect of the nitrogenous substances, with following the changes of total nitrogen and protein nitrogen and were not concerned with the partition of the total nitrogen into that of individual proteins and simple nitrogenous substances. The same remark applies to a later and similar investigation by Thatcher [3] into the progressive development of the wheat kernel. More detailed information is necessary, however, if a comprehensive picture of the nitrogenous metabolism of the wheat plant is to be obtained.

The investigations of Osborne and his co-workers [4] have established with certainty that there are at least five forms of protein present in the wheat kernel. These comprise: (1) Gliadine, distinguished by its ready solubility in alcohol of strength 70 per cent. by volume; (2) Glutenine, soluble in very dilute acids and alkalies, but insoluble in dilute alcohol and neutral aqueous solvents; (3) Leucosin, an albumin-like protein, easily soluble in distilled water; (4) a globulin, soluble in dilute saline solutions; (5) a proteose, soluble in water.

The albumin, globulin and proteose constitute together almost the entire protein of the embryo, whilst the gliadine and glutenine form nearly the whole of the protein of the endosperm, or more than 80 per cent. of the total protein of the entire seed. The following table gives Osborne's figures for the distribution of these proteins in the whole wheat kernel.

	Spring wheat %	Winter wheat %
Gliadine	3·96	3·91
Glutenine	4·68	4·17
Albumin	0·39	0·36
Globulin	0·62	0·63
Proteose	0·21	0·43

The above figures however are subject to modification according to the variety of wheat under investigation.

In addition to the forms of nitrogen enumerated above, the wheat grain also contains nitrogenous substances of non-protein type, although the present knowledge of such substances and their functions in the kernel is incomplete. Schulze (5) concluded that the amount of non-protein nitrogen in the wheat kernel amounted to 0·24 per cent. (calculated on the dry matter basis). He demonstrated the presence of a little asparagine in the wheat embryo, but stated that the amount was insignificant when expressed as a percentage of the whole grain. Richardson and Crampton (6) noted the presence of allantoin in the embryo, whilst Frankfurt (7) was able to identify the bases betaine and choline amongst the constituents of the embryo. Blish (8) showed that patent wheat flour contained about 2 mg. of amino acid nitrogen and 6·0 mg. amide nitrogen per 100 gm. of flour, and concluded that there was probably a relatively significant amount of non-protein nitrogen of undetermined nature present which was neither in the form of amino acid nitrogen nor of peptide complexes. Jodidi and Markley(9), as a result of careful investigations on the whole wheat grain, demonstrated the presence of significant amounts of amino acid nitrogen (1·4 to 2·3 per cent. of the total nitrogen) and amide nitrogen (1·5 to 1·9 per cent. of the total nitrogen) and for the first time established the important physiological fact that the kernel also contained polypeptide nitrogen (3·9 to 5·1 per cent. of the total nitrogen). In addition to the above, the seed of the wheat has also been shown to contain nucleic acid (probably as nucleates in the embryo), phytosterol, lecithin and traces of arginine.

Although the main object of the present investigation centred round the nitrogenous constituents of the grain, the opportunity was also taken to follow quantitatively the changes in the moisture content, crude fat, crude fibre, inorganic constituents and acidity of the aqueous extracts. No quantitative measurements of the amount of reducing sugars were made, the changes in the reducing power of the grain extracts being followed by qualitative tests carried out under comparative conditions.

GENERAL ARRANGEMENT OF INVESTIGATION

For the purposes of the investigation a rigidly preserved pure line of Red Fife wheat was selected as one which was likely to yield a grain of satisfactory strength, containing a fair percentage of protein, this latter fact rendering the variety suitable for investigation of the development of the nitrogenous constituents. It was sown on Nov. 6, 1922, on

a uniform light gravel soil which had been lightly dressed with guano in the autumn. The rate of sowing was one grain per hole at 2 inch intervals, whilst the distance between successive rows was 6 inches.

The crop grew satisfactorily and was not lodged. It was almost free from disease, a few isolated plants being affected by yellow rust (*Puccinia glumarum*) and bunt (*Tilletia tritici*). These were carefully excluded from the samples taken for analysis.

The analytical samples consisted of the grain in the ears from the central stems of 120 plants (note: in the earlier stages the samples were obtained by removing the grain from 200 such ears). The ears were selected on the simple principle of deciding by eye judgment the representative stage of ear development over the whole plot and carefully choosing the requisite number of ears which had arrived at this stage. To such a method of sampling there is necessarily the objection that formal precautions to ensure uniformity are lacking.

Amongst individual grains from a population of wheat plants there is at every stage fluctuation within wide limits. This has primarily a three-fold basis: (1) The individual plants do not pass synchronously or similarly through the outwardly marked stages of development. (2) For any one plant the separate tillers represent in time and magnitude a more or less graded series. (3) On the individual ear, the florets and the grains they form are not uniform but complexly graded.

Precautions might be devised to cope, theoretically, with these three basal forms of fluctuation. It has been demonstrated, however, that only by the most elaborate safeguards can all the theoretical implications be met and even with very careful safeguarding, high uniformity in sampling is still unattainable (Engledow and Wadham, 1923 and 1924) [10].

With cereal plants sampling difficulties spring largely from the fact that of a number of attributes, each a potential index to the stage of development, no two give results entirely in harmony. In view of the nature of the objects of this investigation, it was considered desirable to confine attention to major and outstanding features as a guide to sampling, since elaborations in this connection, whilst having only limited value, would add greatly to the labour involved in the investigation. At some stage or other of cereal investigation, reliance must ultimately be placed in eye judgment. Properly employed, it often affords the best basis for sampling and at times the only one. Usually, as in the present trial, the sample to sample sequence of analytical figures affords a critical check on the adequacy of the sampling procedure.

On the sampling days, the selected ears were cut at about 9 a.m. and transported without delay to the laboratory, where the grain was removed forthwith by a team of workers. The grain as it was removed was stored in a stoppered bottle to prevent loss of moisture and was weighed prior to analysis to ascertain the average weight of kernels per ear. The analysis of the sample was proceeded with immediately, so that no part of the grain remained in a changeable condition for more than about two hours after cutting the ears.

The first sample was taken on July 9, 1923; a second sample was investigated a week later and thereafter biweekly samples were collected up to harvest.

No meteorological data are published nor is there any attempt to correlate the chemistry and chronology of development with environment and weather conditions. It was noteworthy that, save for a heavy storm from which the plot escaped undamaged, the weather from ear-emergence to harvest was very favourable to grain development and maturation. To this is no doubt in some measure to be attributed the regularity of sequence which the analytical data display. Of the influence of the weather, however, no more can be said. The effect of a climatic factor is not simply determined by its intensity and duration; its precise incidence in plant-physiological time must be fundamentally important. Moreover, climatic effects on plants may not be revealed at once, but may only become manifest by reason of their influence on a later phase of plant activity. The certainty of strong pre-determinative effects in the reaction of plants to weather points to the futility of considering formal meteorological data in an investigation of this kind.

BOTANICAL NOTES.

No cytological investigation was made in connection with the chemical studies to be recorded. Careful notes of size and appearance of the grain, however, as well as of developmental stage of the complete plant, were made at every sampling. These data, together with actual dates of sampling, enable the main trend of cytological development to be indicated in an approximate sense.

Several detailed records of the development of the wheat plant embracing cytology, morphology and chronology have been published (Brenchley (11), Percival (12)). In the outline which follows, actual observations are coupled with facts borrowed from published data and inserted in appropriate chronology. Zero day is taken as the modal day of ear-

emergence for the whole plot. Fig. 1 shows in schematic form the time position of the analytical samples.

The times of ear-emergence, anthesis and fertilisation are shown in Fig. 1. The first division of the fertilised ovum would occur between the 10th and 12th days and by the 13th day the formation of endosperm cells would be actively in progress.
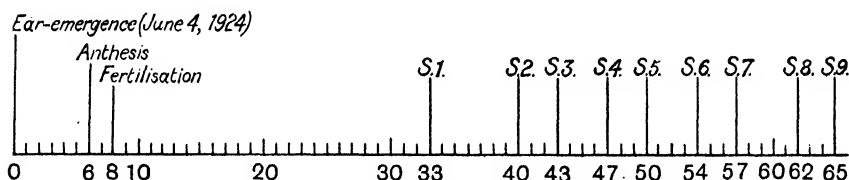
Fig. 1. Numbers along the base line indicate days from time of ear-emergence.
S. 1 implies Sample 1 and so on.

By about the 21st day the full number of endosperm cells would be formed. At this stage the aleurone layer would be visible in a section and the infiltration of starch into the endosperm cells about to begin. Some nine or ten days later the upper endosperm cells would already be filled with starch and the first leaf of the embryo would be differentiated. After a further five or six days the first pair of primary rootlets would be observable.

On the 33rd day, sample 1 was collected for analysis. The grains were about half their final maximum length and quite milky. Though not green in external appearance, they contained sufficient pigment in the chlorophyllous integumentary layer to colour the grain extracts.

Sample 2 was taken on day 40, when the grains were three-quarters the full length. In consistency they were between milky and cheesy. They appeared definitely green externally.

Sample 3 on the 43rd day showed only slight progressive differences from sample 2.

Sample 4 on day 47 consisted of grains of the maximum length (length decreased from this time on). In consistency they were almost cheesy and their external colour was just perceptibly tinged with yellow.

Sample 5 on day 50 marked an important change, for the external green colour was quite noticeably tinged with yellow-brown. At this stage all the endosperm cells contain abundant starch and infiltration of the latter is almost finished.

Sample 6 on day 54 consisted of semi-hard grains definitely brown-green in external appearance. By this time the disintegration of the

endosperm cell nuclei, induced by the pressure of starch infiltration, must have commenced.

Sample 7 on day 57 and sample 8 on day 62 possess no special cytological interest. The crop was ripe to harvest on day 65, when the final sample was taken.

Botanically, it is of interest to note the evidences of correspondence between chemical and cytological phases, this being particularly marked in sample 5.

METHODS OF ANALYSIS.

Dry matter of grain. Duplicate samples of about 20 gm. of the grain were dried in the steam oven to constant weight.

Total nitrogen. Determined by means of Kjeldahl method on duplicate samples of fresh grain.

Acidity of aqueous extracts. A representative sample of 25 gm. of the fresh grain was weighed into a small beaker. After adding a little distilled water, the beaker was plunged for five minutes into boiling water. By this means the possibility of proteolytic enzyme activity affecting the distribution of the nitrogenous constituents during extraction was obviated. The grain was next transferred to a mortar and very thoroughly ground up under distilled water. The liquid was then poured off through a filter into a graduated flask. The process of grinding up the residue under distilled water was repeated several times, until the volume of clear filtrate amounted to 300 c.c. After thorough mixing, 50 c.c. of the aqueous extract was titrated with $N/10$ NaOH to phenolphthalein.

Amino acid and ammonia nitrogen. 175 c.c. of the clear aqueous extract obtained in the determination of acidity as above was made up to 500 c.c. with neutral alcohol. After shaking and allowing the precipitated material to settle, the liquid was filtered and the amino acids (including asparagine) and ammonia in the alcoholic filtrate were determined by the use of Foreman's method [13].

Nitrogen extracted by neutral salt solution. A weighed sample of the fresh grain was ground up thoroughly with 1 per cent. NaCl solution in a mortar. The extract was filtered into a graduated flask. The process of extraction of the residue was repeated many times, until the volume of the extract amounted to 300 c.c. In order to ensure thorough removal of soluble constituents, the residue, after the final grinding up with salt solution, was thoroughly pressed out in a small linen bag. The saline extract was next thoroughly mixed and nitrogen determinations were

carried out on 100 c.c. portions. The weight of grain thus extracted was 25 gm. in the earlier and 20 gm. in the later samples.

Alcohol soluble nitrogen. The residue from the determination of the saline soluble nitrogen was next carefully washed back into the mortar by means of alcohol (70 per cent. by volume) and was thoroughly ground up with several successive portions of alcohol of this strength, the alcoholic solution being filtered off into a measuring flask after each extraction. This treatment was followed by shaking the residue further with 70 per cent. alcohol in a flask at intervals during 24 hours, the liquid being filtered off and added to the bulk of the previous filtrates. The extraction was continued in this manner until no further gliadine was dissolved (shown by pouring a little of the extract into distilled water and noting absence of turbidity). The total volume of alcoholic filtrate obtained in the determinations at the earlier stages was 250 c.c., whilst with the later samples it was found necessary to increase the bulk to 500 c.c. After thorough mixing, 100 c.c. of the extract was pipetted into each of two digestion flasks, the alcohol evaporated off in the water bath and the nitrogen in the residues determined by the Kjeldahl method.

Alkali soluble nitrogen. The material remaining after extraction with 70 per cent. alcohol was allowed to air-dry and was then shaken vigorously in a flask at frequent intervals during a period of 48 hours with a measured volume (250 c.c. with the early samples and 500 c.c. in the later determinations) of 0·2 per cent. KOH (with the addition of a little toluene to prevent bacterial action). The alkaline extract was then filtered clear and the nitrogen content determined in duplicate on 100 c.c. samples.

True protein and non-protein nitrogen. The grain remaining after weighing out for the determinations described above was dried in the steam oven and subsequently finely ground up. The material was then air-dried and, after determination of the moisture content, the percentage of true protein nitrogen was determined by means of the copper precipitation method. This was subtracted from the total nitrogen, the difference representing the amount of non-protein nitrogen in the grain.

Inorganic constituents. Ash determinations were carried out in the usual manner on the air-dried material. Difficulty was experienced, however, in obtaining a perfectly white ash and this was overcome in all cases by heating gently the grey ash with a few drops of concentrated nitric acid, followed by ignition at low red heat.

Crude fat and acid values of crude fat. Determinations of crude fat were carried out on the air-dried material by the usual Soxhlet extraction method. In order to determine the acid values of the ether extracts,

weighed amounts of the material were extracted with ether for three days. After removing the ether at a low temperature, the residue was forthwith dissolved in a hot neutral alcohol-ether mixture and titrated with $N/10$ NaOH to phenolphthalein.

Crude fibre and carbohydrates. The crude fibre content of the grain meal was estimated by the orthodox method. The carbohydrates were calculated by the difference method and for this purpose it was necessary to assume the factor 5·7 for converting total nitrogen into crude protein.

All the results so obtained were subsequently calculated so as to relate to 100 gm. of the dry grain.

Reducing sugars and nitrate. No quantitative estimations of starch and reducing sugars were made. A comparative qualitative examination of the samples of grain was carried out in the following manner. 3 gm. of the air-dried samples was shaken for one hour with 30 c.c. distilled water. After filtering clear, 5 c.c. of the filtrates was boiled with 2 c.c. of the mixed Fehling's reagent and the readiness and copiousness of the reduction were noted.

Tests for the presence of nitrates were also carried out on the aqueous extracts, employing both the carbazole and diphenylamine reagents. In making this test, the reagents were not only added to samples of the grain extracts, but also to separate portions of the extracts to which had been added two drops of KNO_3 solution (1 part in 10,000). In this manner, it could be ensured that the test was being carried out under conditions which would enable a positive result to be noted if small amounts of nitrates were present in any of the extracts. That the conditions were such was shown by the fact that decided positive results were obtained in every case where grain extract was tested after the addition of the two drops of dilute KNO_3 solution.

CRITICAL SURVEY OF ANALYTICAL METHODS.

It cannot be claimed that the above scheme of analytical methods is wholly satisfactory, nor even that the nitrogenous fractions so determined represent absolutely sharp and distinctive types of nitrogenous substances. Bailey and Blish [14], for instance, have demonstrated that the extract of flour when 1 per cent. NaCl is employed as solvent does not only contain the non-gluten proteins, but also measurable amounts of gliadin. They also call attention to the difficulty of extracting completely the gliadin by means of 70 per cent. alcohol at the ordinary temperature. In dealing with the complex system of embryo, aleurone

layer and endosperm, other than aleurone, it is entirely probable that any attempt to isolate the various proteins by the simple use of solvents will result in some overlapping.

The fraction of nitrogen extracted by means of dilute NaCl solution will comprise not only that of the leucosin, globulin and proteose constituents, but also the nitrogen contained in the simple nitrogenous substances such as amino acids, amides and ammonium salts. No attempt was made to estimate the albumin, globulin and proteose fractions separately, as such an addition to the experimental procedure would have rendered the analytical routine too laborious to be carried out smoothly. It is proposed to carry out a further investigation for the purpose of following in detail the behaviour of the water soluble constituents during grain development.

The method whereby the glutenine was estimated in the whole wheat meal was perforce of an unsatisfactory nature, since it involved extracting with dilute alkali the residue after removal of saline soluble and alcohol soluble nitrogenous constituents. It cannot therefore be asserted with certainty that glutenine, and glutenine alone, was extracted in this process.

No serious criticism can be urged against the methods whereby the dry matter, total nitrogen, protein nitrogen, crude fat, crude fibre and ash were estimated. ·

It should be pointed out that the amino acids as determined by Foreman's method include also amides of the asparagine and glutamine type, since the latter are titratable in alcoholic solution. Preliminary experiments carried out on pure asparagine showed that the latter was perfectly stable during the operation employed for the ammonia estimations.

The total carbohydrates were calculated by the usual difference method, this involving the assumption of the value 5·7 as representing the factor for converting total nitrogen into protein at all stages of the grain's development. No attempt was made to differentiate the carbohydrates quantitatively into starch, dextrin, reducing sugars, hemicelluloses, etc.

It would be difficult if not impossible in the present state of knowledge to evolve an analytical procedure for this purpose which would be free from criticism and at the same time be practicable from the point of view of time and labour involved in its carrying out. The methods which were actually adopted, however, enable the grain contents to be partitioned at every stage into a series of comparable fractions and thus

Table I. Analysis of samples of grain (calculated on dry matter basis).

Number of sample	1	2	3	4	5	6	7	8	9
Days from ear-emergence	33	40	43	47	50	54	57	62	65
	%	%	%	%	%	%	%	%	%
Dry matter	27·54	37·77	44·80	50·62	54·99	59·56	64·79	77·18	83·54
Total nitrogen	2·56	2·48	2·44	2·59	2·67	2·70	2·69	2·80	2·78
Protein nitrogen	1·75	2·10	2·11	2·04	2·25	2·50	2·44	2·56	2·58
Non-protein nitrogen	0·81	0·38	0·33	0·55	0·42	0·20	0·25	0·24	0·20
Nitrogen soluble in NaCl solution	1·77	1·00	0·87	0·76	0·68	0·62	0·53	0·55	0·52
Gliadine nitrogen	0·15	0·42	0·78	0·80	0·79	0·91	0·90	1·02	1·02
Glutenine nitrogen	0·15	0·26	0·36	0·44	0·58	0·54	0·58	0·54	0·56
Amino acid nitrogen	0·413	0·109	0·076	0·071	0·064	0·039	0·038	0·020	0·021
Ammonia nitrogen	0·508	0·091	0·059	0·163	0·137	0·139	0·102	0·111	0·111
Nitrate nitrogen	—	—	—	—	—	—	—	—	—
Crude fat	2·12	2·65	2·69	2·83	2·45	2·29	2·29	2·27	2·21
Crude fibre	4·26	3·59	2·93	2·36	2·41	2·36	2·34	2·32	2·32
Ash	3·06	2·31	2·17	1·93	1·69	1·70	1·65	1·68	1·67
Carbohydrates (by difference)	75·90	77·31	78·30	78·12	78·23	78·26	78·39	77·77	77·95
Acid values (mg. KOH per gm. fat)	90·0	69·1	49·5	47·3	33·3	20·9	18·4	19·0	18·5
Acidity of aqueous extract (c.c. N)	12·7	4·0	5·1	4·1	4·1	4·0	2·8	1·6	2·3

Table II. Showing amounts of constituents calculated on basis of grains in 100 ears.

Number of sample	1	2	3	4	5	6	7	8	9
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fresh weight	85·2	198·0	242·4	267·0	281·0	255·0	242·4	201·3	185·0
Dry matter	23·5	74·8	108·6	135·2	154·5	151·9	157·1	155·4	154·6
Water	61·7	123·2	133·8	131·8	126·5	103·1	85·3	45·9	30·4
Total nitrogen	0·60	1·86	2·65	3·50	4·13	4·10	4·23	4·35	4·30
Protein nitrogen	0·41	1·57	2·29	2·76	3·48	3·80	3·83	3·98	3·99
Non-protein nitrogen	0·19	0·29	0·36	0·74	0·65	0·30	0·40	0·37	0·31
Nitrogen soluble in NaCl solution	0·42	0·75	0·94	1·03	1·05	0·94	0·83	0·85	0·80
Gliadine nitrogen	0·04	0·31	0·85	1·08	1·22	1·38	1·41	1·59	1·58
Glutenine nitrogen	0·04	0·19	0·39	0·60	0·90	0·82	0·91	0·84	0·87
Amino acid nitrogen	0·10	0·08	0·08	0·10	0·10	0·06	0·06	0·03	0·03
Ammonia nitrogen	0·12	0·07	0·06	0·22	0·21	0·21	0·16	0·17	0·17
Crude fat	0·50	1·98	2·92	3·83	3·78	3·48	3·59	3·53	3·42
Crude fibre	1·00	2·69	3·18	3·19	3·72	3·59	3·67	3·61	3·59
Ash	0·72	1·73	2·36	2·61	2·61	2·58	2·59	2·61	2·58
Carbohydrates (by difference)	17·85	57·82	85·03	105·62	120·87	118·88	123·15	120·85	120·51

make possible the investigation in a broad sense of the progressive changes which characterise, from a chemical point of view, the growth of the grain.

<div align="center">DISCUSSION OF ANALYTICAL RESULTS.</div>

<div align="center">1. *Fresh weight, dry matter and water content.*</div>

Brenchley and Hall[2] broadly divided the period of development of the wheat grain into three periods, basing the division on the changing water content of the grain. (1) The period comprising about 22 days after flowering in which the amount of water in the grain is rising continuously. (2) A further period of about 18 days during which the water content of the grain remains roughly constant. (3) A final ripening period of about 6 days during which the grain is being rapidly desiccated. The critical days in the history of the growth of the grain were about 22 and 40 days after flowering.

A study of the data given in Table II reveals the fact that the growth period of the grain examined in the present investigation can similarly be divided into three such periods. Up to day 40 after ear-emergence the water content of the grain shows marked rises; from day 40 to day 50 the water content is roughly constant, whilst from day 50 to day 65 a rapid desiccation takes place. The critical days of the present trial were therefore day 40 and day 50 after ear-emergence.

It will be noted that the "constant water content" period was only of 10 days' duration, whereas in the investigations of Brenchley and Hall, the corresponding period was of about 18–20 days in length. It is probable therefore that although the growth of the grain can be divided into three periods showing the characteristic features of rising water content, constant water content and desiccation, the relative durations of these periods may be in a measure dependent on variety of wheat and conditions of climate and soil.

The green weight of the grains from 100 ears increased continuously until a maximum was reached on the critical day 50; from this point onwards the green weight diminished regularly. It has already been pointed out (see Botanical Notes) that day 50 was marked by the setting in of the change of the external colour of the grain from green to yellow-brown.

The percentage of dry matter in the grain showed a steady increase right up to harvest. On the other hand, the actual dry weight of the grain per 100 ears increased rapidly up to day 50, and beyond this the ripening period was characterised by only slight dry weight changes. The figure

151·9 gm. for sample 6 is probably on the low side as a result of accidental errors of experiment. There is every indication that the actual maximum dry weight was attained on day 57, *i.e.* about a week before the crop was considered ripe to harvest. In the last week a slight but measurable diminution in dry weight occurred. This is in agreement with the findings of Brenchley and Hall and is probably to be attributed to loss of carbohydrate resulting from respiration at a stage when no further nutrients are being moved up into the grain.

Table II brings out strikingly the character of the ripening process, which is almost exclusively one of desiccation, unaccompanied by transport into the grain of fresh material or the occurrence of any marked chemical changes. Rearrangement of material already in the grain, such as the transformation of non-protein nitrogen into protein, may occur to a minor extent at the beginning of desiccation.

This feature has already been amply demonstrated by the investigations of Brenchley and Hall. It is difficult therefore to understand the results of Eckerson (15), who, as a result of microchemical studies, concluded that the synthesis of endosperm proteins took place during the desiccation period.

2. *Total nitrogen, protein nitrogen and non-protein nitrogen.*

The percentage of total nitrogen on the dry matter did not display much variation during the whole period of development. In the first 10 days, the percentage fell slightly; thereafter it rose from 2·44 per cent. to about 2·70 per cent. The further slight rise experienced during the last week of ripening is probably explained by the loss of non-nitrogenous material from the grain by respiration rather than actual gain of nitrogenous material by transport. It is to be noted that the final percentage of nitrogen in the mature grain is greater than that in the immature samples.

It is of interest to contrast this result with that obtained by André in his investigations on the barley grain (16). André showed that the barley grain does not possess a maximum content of nitrogen at the end of ripening but at an earlier stage, apparently for the reason that considerable amounts of nitrogen-free materials are stored up later. At the end of ripening, barley contains about 16·4 per cent. less nitrogen than at the maximum phase.

In the present investigation, the amount of nitrogen in the grains from 100 ears rose regularly up to the critical day 50, the rise after this day during the ripening period being very small.

The percentage of protein nitrogen on the dry matter rose somewhat irregularly from 1·75 per cent. up to 2·58 per cent. during the experiment, whilst that of the non-protein nitrogen, which was highest in sample 1, fell from 0·81 per cent. to 0·20 per cent. in the mature grain. The latter figure agrees well with the average figure of 0·24 per cent. given by Schulze (5) for the non-protein nitrogen in the dry matter of the ripe wheat kernel.

The gradual alteration in the character of the nitrogen of the grain is shown in Table III, which gives the protein nitrogen expressed as percentages of total nitrogen for the different samples.

Table III.

Number of sample	1	2	3	4	5	6	7	8	9
% protein N in total N	68·4	84·7	86·5	80·0	84·3	92·6	90·7	91·4	92·8

The amount of protein nitrogen in the grain from 100 ears rose steadily and fairly regularly up to day 50. In the next four days a further small rise in the amount of protein nitrogen occurred, this obviously resulting from the building up of protein from non-protein nitrogen already present in the grain at this stage, since the non-protein nitrogen underwent a diminution almost equal to the rise in protein nitrogen. It follows therefore that the conversion of non-protein nitrogen into protein is possible on a reduced scale at the beginning of the desiccation period, when transport of further material into the grain has ceased.

The amount of non-protein nitrogen in the grain from 100 ears showed a steady increase up to day 47 after ear-emergence, this pointing to the conclusion that up to this stage nitrogen in the form of simple substances was being transported into the grain at a greater rate than it could be built up into protein. After day 47, when transport of nitrogen into the grain was beginning to slow down, the amount of non-protein nitrogen in the grain from 100 ears began to show a decrease also. After day 50, transport into the grain had ceased whereas synthesis of protein continued for a few days longer. This point was marked therefore by a decided fall in the amount of non-protein nitrogen. After day 54 the grain was too dry to permit of both synthesis and transport, so that the relative amounts of protein and non-protein nitrogen remained roughly unaltered up to harvest.

3. *Amino acid (including asparagine), ammonia and nitrate nitrogen.*
Discussion of mechanism of protein synthesis in the grain.

In sample 1, where the percentage of non-protein nitrogen on the dry matter possessed the highest value, the whole of this fraction could be accounted for in terms of amino acid (including asparagine) and ammonia nitrogen. The slight discrepancy which occurred was obviously to be attributed to experimental error involved in the measurements. At every other stage, the sum of the amino acid (including asparagine) and ammonia nitrogen was definitely smaller than the amount of non-protein nitrogen. This points to the appearance in the grain of non-protein nitrogenous substances which cannot be included in these two classes, such as "true amides" (*i.e.* amides which unlike asparagine do not contain a carboxyl group), nucleic acid, lecithin, phytosterol, etc.

The amount of amino acid (including asparagine) nitrogen in sample 1 represented about 16 per cent. of the total nitrogen. A week after this the percentage dropped to about 4·4 and thereafter continued to fall, so that in the mature grain the amino acid (including asparagine) constituted only about 0·75 per cent. of the total nitrogen.

There can be no question of amino acids accumulating in the immature grain and subsequently being condensed to form protein during the desiccation period, as has been suggested by Eckerson [15]. The synthesis of protein from amino acids is proceeding at all stages up to the desiccation period in a continuous manner, and it is this fact which explains the small concentration of the amino acids in the grain during the "constant water content" stage of development. As amino acids appear in the grain, they are more or less promptly transformed into more complex forms of nitrogen.

The question arises as to the nature of the nitrogenous materials which immediately precede the amino acids in the chain of reactions involved in the synthesis of the seed proteins. The results of the analysis of sample 1 appear to afford some clue to this. Here the whole of the non-protein nitrogen may be partitioned into amino acids, amides of the asparagine type and ammonium compounds. It will be noted that in this sample, ammonia nitrogen was present in somewhat larger amount than amino acid (including asparagine) nitrogen. From the fact that at no investigated stage in the development of the grain could nitrate be detected by the most sensitive tests, it is justifiable to conclude that the reduction of nitrate to ammonia (probably through nitrite) occurs at some point exterior to the grain and that the nitrogen for protein

synthesis enters the grain either in the form of amide (asparagine) or ammonium compounds, or both.

Modern research has assigned to asparagine an important rôle in connection with the nitrogenous metabolism of the plant. Various earlier workers noted that whenever the amount of protein in leaves underwent diminution, there was a corresponding appearance of asparagine (Borodin [17], Schulze [18], Butkewitsch [19]). Mercadente [20] put forward the opinion that asparagine was the nitrogenous substance concerned in the elaboration of protein in the plant. Schulze [21], whilst admitting the possibility of synthesis in this manner, doubted whether it occurred as a result of direct combination between the amide and nitrogen-free materials.

The more recent researches of Prianischnikoff and of Chibnall have brought out clearly the part which asparagine plays in plant metabolism. Prianischnikoff and Schulow [22] expressed the view that asparagine synthesis occurred in the plant whenever an excess of ammonia was present in the tissues, arising from too quick uptake of nitrogen through the roots or from deamination changes in the plant itself. In a later communication, Prianischnikoff [23] developed an analogy between the functions of urea in the animal organism and asparagine in the plant. He demonstrated that when in young plants protein metabolism gives rise to ammonia, the latter, which is toxic in excess, is rendered innocuous by condensation with aspartic acid to form asparagine. Chibnall [24] has further shown that the same reaction may occur in the mature leaf under abnormal circumstances. Plants therefore remove ammonia as asparagine, whilst the animal organism renders it harmless by converting it into urea.

The analogy is not perfect, however, since urea is excreted unchanged from the animal organism, whereas in the plant, asparagine may accumulate without harmful effects and may, in the presence of fresh supplies of carbohydrate resulting from carbon assimilation, be further utilised and its nitrogen be built up into protein. Prianischnikoff showed that in seedling plants the amount of asparagine formed is dependent on the amount of available carbohydrate. Amino acids and protein were formed from asparagine in the presence of carbohydrates and in view of the reversibility of this reaction, it followed that the absence of carbohydrate led to the degradation of protein with asparagine formation.

Chibnall [24] has shown that the total amount of protein present in runner bean leaves is subject to a very decided diminution during the night. Moreover, the products of protein degradation, whatever their

nature, do not accumulate in the leaf but are translocated to other parts of the plant.

In a second communication, Chibnall(25) has further demonstrated that asparagine is the chief product of the metabolism of protein in the mature leaf, and he arrives at the conclusion that in the normal mature leaf there is a continuous production of asparagine from protein degradation and this constitutes the most important means at the plant's disposal whereby "nitrogen in a form suitable for easy resynthesis of protein is conveyed from one part of the mature plant to another."

Similarly, during the ripening of seeds, asparagine is probably translocated from the leaves to the pericarp and it is there utilised for the synthesis of seed proteins (Schulze, 1911)(26).

The investigations summarised above point to the dual function of asparagine in the plant organism. Firstly, it is to be regarded as a means of storing ammonia in an innocuous form, and this function probably explains its presence in roots and tubers, the nitrogen which has been taken up in excess of the plant's requirements having been stored as asparagine for future use. Secondly, it possesses the function of a translocatory substance (i.e. an "ammonia carrier") and in this rôle is responsible for the transport of nitrogen from one part of the plant to another for purposes of protein synthesis.

A third function possessed by asparagine is apt to be lost sight of, namely, its power of condensing with amino acids to become an integral part of a protein molecule, since asparagine is probably present as such in the protein molecule, giving rise to aspartic acid on acid hydrolysis.

There can, in the light of the above, be little doubt that the nitrogen for protein synthesis enters the wheat kernel mainly in the form of asparagine arising from degradation of protein in the leaves of the plant. Little or no evidence however is available to show how the further reaction between asparagine and carbohydrate to form protein takes place. That a direct action occurs is extremely doubtful, and a study of the data in Tables I and II lead to the belief that the ammonia is regenerated from the asparagine molecule and that ammonia forms the actual starting point of protein synthesis by entering into reaction with carbohydrate or transformation products of carbohydrate. In sample 1, the amount o ammonia nitrogen is so significant, that it is justifiable to conclude that either a part at least of the nitrogen enters the grain in this form or that ammonia is split off from asparagine previous to its transformation into amino acid nitrogen.

The amount of ammonia nitrogen in the grain at any stage will

be regulated by a two-fold consideration: (1) the rate of its appearance in the grain, either by direct transport or regeneration from asparagine; (2) the rate of its conversion into amino acids or the precursors of amino acids.

In the earliest stage investigated, the amount of ammonia nitrogen in the grain was somewhat greater than that of the amino acid (including asparagine) nitrogen. Between days 33 and 43, the reverse held true, whilst from day 43 onwards the ammonia nitrogen again appeared in excess. Owing to the fact that Foreman's volumetric determinations do not distinguish between asparagine and amino acids, it is unfortunately not possible to interpret with certainty these relationships.

The study of the amounts of the various constituents in the grain after day 50, during the desiccation period, is in the writers' opinion, of importance, since here chemical changes have almost ceased and whatever nitrogenous substances are found present even in small amount may be assumed to be fractions left over from the processes of protein synthesis and "fixed" in the dry grain. Thus the study of the mature grain must afford a distinct clue to the nature of the nitrogenous metabolic changes which go on in the immature stages and the facts cited in the introduction to this communication assume a new significance when considered from this point of view. It will be noted that in the desiccation period, the amount of ammonia nitrogen in the grain definitely exceeded that of the amino acid (including asparagine) nitrogen.

If, as appears probable, the ammonia is regenerated from asparagine previous to further change, the question arises as to the manner in which it is built up into protein. It is interesting to recall in this connection Liebig's [27] early suggestion that protein synthesis in the plant arises from the interaction of ammonia and sugar. Delbrück [28] discovered that yeast cells developed rapidly when they were placed in a solution containing only ammonium sulphate and sugar. It cannot be doubted, however, that the mechanism of protein synthesis is much more complicated than would be suggested by such simple interaction.

One theory which has obtained loose acceptance suggests that the ammonia combines with organic acids in the plant and that the resulting ammonium salts pass with loss of water into amides. The latter are then by some unexplained mechanism transformed into amino acids.

Whilst, however, the figures obtained in this and other investigations (compare Chibnall, 1924) do not preclude the possibility of the existence of "true amides" in the plant, yet it must be pointed out that asparagine and glutamine (intermediate between "true amides" and amino acids)

are the only members of this class which have been isolated with certainty. It is further difficult to visualise the mechanism whereby an amide of the nature of CH_3CONH_2 can pass into an amino acid of the type CH_2NH_2COOH, such a change involving the transference of the NH_2 group from one carbon atom to another. It is certain that such a change could not be brought about in a simple manner.

It is much more probable, though direct experimental confirmation appears to be lacking, that amino acids in the plant are formed at least in part by a reversal of the normal deamination processes. The reaction of ammonia with α-hydroxy acids arising from carbohydrate metabolism might give rise to α-amino acids. In this way, alanine might be formed from lactic acid in the plant.

$$CH_3CHOHCOOH + NH_3 = CH_3CHNH_2COOH + H_2O$$

Lactic acid   Alanine
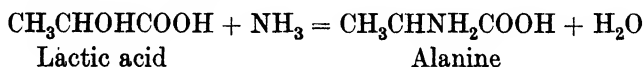
Erlenmeyer and Kunlin [29] were able to effect the synthesis of formyl glycine by the action of ammonia on glyoxylic acid, which is known to occur in plants.

A further possibility in connection with the production of amino acids in the plant lies in the addition of ammonia to unsaturated organic acids. Engel [30] obtained aspartic acid by the action of ammonia on fumaric acid, whilst Fischer and Raske [31] converted β-vinyl acrylic acid into diaminovaleric acid.

It is also not difficult to draw up a scheme illustrating the possible method whereby tyrosine is formed in the plant by the addition of ammonia to p-hydroxycinnamic acid in the following simple manner:

$$OHC_6H_4CH:CHCOOH + H.NH_2 \longrightarrow OHC_6H_4CH_2CHNH_2COOH$$
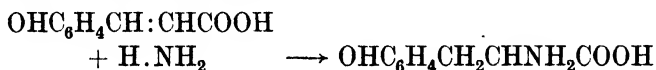
Such a hypothesis would presuppose the presence of p-hydroxycinnamic acid in the plant. This is by no means impossible, since o-hydroxycinnamic acid is known to be distributed in certain plants giving rise to the lactone coumarin, which is responsible for the sweet odour of woodruff, the tonka bean and new-mown hay. In the absence of direct experimental confirmation, however, such reasoning as the above must remain somewhat speculative.

The changes whereby amino acids are condensed to form protein are not difficult to formulate. Nedokutschajew [32] has demonstrated the presence of proteoses in the immature grain and Osborne [4] has also proved their presence in the ripe kernel. Jodidi and Markley [9] have

further shown that the wheat grain contains polypeptides. It follows that protein is built up from amino acids through the normal stages of polypeptides and proteoses, the agencies concerned in the synthesis being the proteolytic enzymes of the grain. As to whether one or more enzymes are involved in the elaboration of the different proteins, or whether the embryo and endosperm proteins are mutually related, must for the present remain uncertain.

4. *Storage of proteins in the grain.* (*Proteins soluble in dilute* NaCl,
gliadine and glutenine.) *Gluten formation.*

With no single sample did the sum of the nitrogen fractions extracted from the grain by successive treatment with 1 per cent. NaCl, 70 per cent. alcohol and 0·2 per cent. KOH account for the whole of the nitrogen. In the last six samples the amount of nitrogen unaccounted varied between 0·59 and 0·69 per cent. (dry matter basis). That this discrepancy did not arise from incomplete extraction of gliadine and glutenine is indicated by the fact that a similar discrepancy occurred with sample 1, in which grain the gluten proteins were only present in traces. The cause is probably twofold: (1) the presence of nitrogenous substances in the grain which are not dissolved out by these solvents; (2) the incomplete rupture of the cells of the outer layers of the grain during grinding up with 1 per cent. NaCl, a fraction of the nitrogen of these layers thus escaping extraction. It is scarcely likely, however, that any significant part of the nitrogen of the embryo or gluten proteins remains undissolved in these processes.

It is of interest to note that a small amount of gliadine and glutenine nitrogen (0·15 per cent. on the dry matter in each case) was present in the immature grain of sample 1. A little of the alcoholic extract of this sample was poured into distilled water, but the turbidity formed by the separation of gliadine was scarcely noticeable. On acidifying carefully a portion of the alkaline extract, a very slight turbidity was produced, thus confirming the presence of glutenine in traces.

In the following samples, the percentages of both proteins on the dry matter increased, but that of gliadine rose much more rapidly than that of glutenine. The alcoholic extract of sample 2 gave an appreciable precipitate of gliadine when poured either into distilled water or absolute alcohol, but it was not until sample 4 was reached that the alkaline extract gave a pronounced precipitate of glutenine on acidifying. Throughout the whole trial, however, gliadine was shown to be present in the grain in appreciable excess of glutenine.

Care was exercised in attempting to detect the stage at which the first signs of coherent gluten formation were to be observed during grinding up of the grain with 1 per cent. NaCl. The first distinct signs were noted in the case of sample 5, corresponding with day 50 after ear-emergence, which has already been shown to denote a critical stage in the development of the grain in marking the beginning of the desiccation period. In sample 6, the glutenous character of the residue after 1 per cent. NaCl extraction was very pronounced.

The first signs of gluten formation therefore corresponded with the commencement of desiccation, when the percentage of gliadine nitrogen had risen to 0·8 and the glutenine nitrogen to 0·58 per cent. (on the dry matter). Thereafter the amount of gliadine nitrogen rose to 1·02 per cent. on the dry matter in the mature grain, whereas the glutenine nitrogen remained roughly constant.

It would therefore appear that the presence of a definite amount of glutenine is necessary in the grain before gluten formation is possible, since in samples 3 and 4, where no evidence of gluten formation was obtained, the percentage of gliadine was almost the same as that in sample 5, the main difference being that the percentage of glutenine was appreciably lower. Since the fraction of nitrogen extracted by 1 per cent. NaCl includes not only albumin, globulin and proteoses but also the simpler forms of nitrogen, it is impossible without making assumptions to follow the changes in the amounts of the embryo proteins at the different stages. Assuming the difference between the NaCl soluble nitrogen and non-protein nitrogen to give a rough measure of the nitrogen of the embryo proteins, it will be noted that the percentages of the latter alter in a different manner from those of either the gliadine or glutenine nitrogen.

Table IV. Showing percentages of NaCl soluble N, gliadine N
and glutenine N (dry matter basis).

Number of sample ...	1	2	3	4	5	6	7	8	9
	%	%	%	%	%	%	%	%	%
NaCl soluble protein N	0·96	0·62	0·54	0·21	0·26	0·42	0·32	0·31	0·32
Gliadine N ...	0·15	0·42	0·78	0·80	0·79	0·91	0·90	1·02	1·02
Glutenine N ...	0·15	0·26	0·36	0·44	0·58	0·54	0·58	0·54	0·56

Table V. Showing amounts of NaCl soluble protein N, gliadine N
and glutenine N in the grains from 100 ears.

Number of sample ...	1	2	3	4	5	6	7	8	9
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
NaCl soluble protein N	0·23	0·46	0·58	0·29	0·40	0·64	0·43	0·48	0·49
Gliadine N ...	0·04	0·31	0·85	1·08	1·22	1·38	1·41	1·59	1·58
Glutenine N ...	0·04	0·19	0·39	0·60	0·90	0·82	0·91	0·84	0·87

Although it would perhaps be unsafe to argue too confidently on the NaCl soluble protein nitrogen figures as so obtained, certain general features appear to be recognisable. In the first sample, the percentage of NaCl soluble nitrogen was much greater than that of either gliadine or glutenine nitrogen, but whereas the percentage of the latter underwent continuous increase up to day 50, that of the NaCl soluble nitrogen showed a corresponding diminution. This behaviour serves to bring out the nature of the gluten proteins as reserve materials, a fact which is further illustrated in Table V. The embryo proteins, on the other hand, do not appear to accumulate continuously in the grain, but appear to permit of further change or breakdown after formation. The question naturally arises as to whether they function as precursors of the endosperm proteins. Further speculation on this point, however, will be withheld until the completion of a further investigation involving a more detailed study of the nitrogenous constituents soluble in dilute NaCl solution.

It will be noted that the proteins of the grain made their appearance in significant amount in the following order: NaCl soluble proteins (albumin, globulin and proteose), gliadine and finally glutenine. Spitzer, Carr and Epple [33] reported a very different result obtained from an investigation of the development of the maize grain. They found that in maize grain the glutelin protein (corresponding with wheat glutenine) appeared first. This was followed by the globulins and albumins, whilst zein (corresponding with wheat gliadine) appeared to be formed last. These findings point to a very sharp distinction between the nitrogenous metabolisms of the grain of maize and wheat.

5. *Crude fat and acid values of crude fat.*

The percentage of crude fat (calculated to dry matter basis) was very little different in sample 1 (2·12 per cent.) and the mature sample 9 (2·21 per cent.), although between days 40 and 50 ("constant water content" period) the value had risen to a somewhat higher level. The actual amount in the grains from 100 ears rose continuously up to about day 50 (from 0·5 gm. to about 3·8 gm.), whereafter the amount fell to about 3·5 gm. and remained roughly the same up to harvest.

A study of the acid values shows that the character of the crude fat underwent continuous alteration, the ether extract of sample 1 having the high acid value of 90·0 (*i.e.* 45 per cent. of acid reckoned as oleic acid). In the ripe sample, the acid value of the fat was only about 19 (*i.e.* roughly 9·5 per cent. of acid calculated as oleic acid). It should be

mentioned however that the acid values were determined on the ether extract of the dried grain (see Critical Survey of Analytical Methods) and the possibility of slight hydrolysis of fat occurring during drying, especially in the earlier and wetter samples of grain, should not be lost sight of.

Phytosterol appears in the grain at an early stage, since the ether extract of sample 1 gave a positive test for this constituent.

6. *Inorganic constituents. Acidity of aqueous extracts.*

The percentage of ash (on the dry matter) decreased uniformly from the value of 3·06 per cent. in sample 1 to 1·69 per cent. in sample 5 (day 50), the value remaining constant during the desiccation period. The amount of ash in the grains from 100 ears increased from 0·72 gm. in sample 1 to 2·61 gm. in sample 4. Thereafter the amount remained constant, indicating that transport of inorganic materials into the grain ceases at the beginning of desiccation.

The values of the acidity of the aqueous extract resembled those of the crude fat acid values in displaying a maximum in the first sample. In sample 2 the acidity had fallen from the high value of 12·7 c.c. N to 4·0 c.c. N (referred to 100 gm. dry grain). From sample 2 to sample 6 the value remained roughly constant, but underwent a further drop in the final stages of ripening.

7. *Crude fibre and carbohydrates.*

The percentage of crude fibre in the grain (dry matter basis) was greatest in the earliest sample, the value showing a progressive decrease during filling of the grain. After day 47, the value remained roughly constant. The amount of fibre in the grain from 100 ears increased up to day 50 and displayed constancy, within the limits of experimental error, during desiccation.

The constancy of the percentage of total carbohydrates (dry matter basis) between days 43 and 57 is very striking, the slight fall which occurred in the last stages of ripening being attributable to respiration effects. The figures in Table III show that storage of carbohydrate was complete at the stage corresponding with the beginning of desiccation. Starch was shown to be present in every one of the samples. Further tests revealed the fact that sample 1 was by far the richest in reducing sugars. The reducing power of the aqueous extracts of the grain fell markedly in sample 2 and was even less pronounced, though still quite definite, in the samples taken during the desiccation period.

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THE CHEMISTRY OF THE STRENGTH
OF WHEAT FLOUR.

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(With Three Diagrams.)

WHEAT owes its enormous importance to certain physical properties which are absent in the flours of other cereals, chief among which is the capacity of making a coherent dough on the addition of water.

Different wheats make loaves of different size and texture, a strong wheat being defined by Humphries and Biffen[1] as one which yields flour capable of making large well-piled loaves. From this definition it is seen that the conditions required of a strong flour are two, the first being that there must be a sufficiency of sugar or other materials available for fermentation and consequent production of gas in the dough, the second, that there must be some substance present in the flour which is capable of retaining a sufficiency of the gas so generated. The constituent of wheat flour in virtue of which its dough possesses this quantity of gas retaining power is the gluten. Many workers have attempted to explain strength in terms of the relative amounts of gliadin and glutenin in the gluten, or on the percentage of gliadin in the flour.

Guthrie[2] considered that flour in which glutenin predominated yielded strong, tough, non-adhesive, and elastic gluten.

Fleurent[3] considered that flour in which gliadin and glutenin were in the proportion of 75 per cent. of the former to 25 per cent. of the latter, gave the best bread making results.

Guess[4] considered that strength was associated with a high percentage of gliadin.

Snyder[5] found that flour to which wheat starch was added or subtracted to the extent of 20 per cent. yielded loaves very little different from those made from the normal flour, and so concluded that it is the quality rather than the quantity of gluten which governs the quality of

the bread.   He also found(6) that the ratio of gliadin to glutenin could vary within wide limits without much difference in the strength of the flour.

Wood(7) thought that the gliadin from strong and weak flours might differ in chemical constitution, but finding that the percentage of amide nitrogen in samples of gliadin extracted from both strong and weak flours were the same, concluded that this was not so.   Wood next directed his attention to the water soluble constituents of the flour, and found that neither the acidity nor the percentage of soluble salts gave figures proportional to the strength of the flours.   He found, however, that the nitrogen and ash-free extract, which would consist chiefly of carbo-hydrates, was roughly proportional to the baker's marks.   The idea of separating strength into at least two factors, that of volume and that of shape, each independent of the other, then seemed to him a good working hypothesis.   He then measured the diastatic capacity of various flours by growing yeast in a water suspension of the flour at 37° C. and measuring the carbon dioxide given off.   By this method he found that there was a general relation between strength, as shown by the baker's marks, and the volume of carbon dioxide evolved during fermentation, thus justifying his conclusion that the capacity of a flour for giving off gas when incubated with yeast and water is the factor which in the first instance determines the size of the loaf.   An interesting paper in this connection is by Baker and Hulton(8) on the enzymic activities of flour. They state that Reychler(9) showed that by treating wheat gluten with dilute acid and salt solutions he obtained liquids which possessed dia-static power.   Baker and Hulton examined several specimens of gluten and found that they readily hydrolysed soluble starch.   They next studied the distribution of diastase in the gluten.   After thoroughly extracting a sample of flour with 70 per cent. alcohol to remove the gliadin the alcoholic solution was evaporated down under reduced pressure at 35–40° C. to remove the alcohol, but the matter which was in solution was without effect on soluble starch.   The residue, which consisted of glutenin and starch, had a rich diastatic power.   Having proved that the amylolytic enzymes will resist alcohol of 70 per cent. strength by digesting flour with such alcohol, and then finding its dia-static power was unaltered they concluded that there is strong pre-sumptive evidence that the glutenin portion of the gluten is that which possesses enzymic activity.

Wood's next paper(10) deals with the second factor in strength, that which determines the shape of the loaf.   The paper deals with the effect

of acids and salts on the coherence of gluten. He found that dilute solutions of acids quickly disintegrated pieces of gluten placed in the solutions, but that salts preserved the coherence of the gluten in acid solutions. Wood therefore concluded that it was the acid and soluble salt content of the flour which determine the coherence of the gluten, and so undoubtedly must have a direct bearing on the power of some flours of making shapely loaves.

Jago[11] finds that 90 times the necessary amount of salt is added to overcome the maximum disintegrating effects of say sulphuric acid, and so it would seem that there must be some inherent property of the gluten itself which determines its strength.

Work on the strength of flour was next undertaken by Woodman[12] who examined the gliadin and gluten fractions of the gluten, using a method of procedure he had previously used for the examination of the corresponding proteins of cow and ox serum, and cows' colostrum and cows' milk. The method has its basis in the initial observations of Kossell[13], and of Dakin[14], that when solutions of protein in dilute sodium hydroxide solution are kept at 37° C. they suffer a progressive diminution in the value of their optical rotary power. This change is attributed by Dakin to a keto-enol tautomerism of the $=\text{CH--CO--}$ groups in the protein complex. If the specific rotations of the solutions are plotted against the time in hours during which the reaction is allowed to proceed, then the readings fall on a perfectly smooth curve. Although two proteins might be quantitatively identical with regard to their amino nitrogen content they might be two distinct proteins by virtue of the differences in order of linkage of the amino acids within the protein molecule. Woodman's work had shown that such differences were shown up by comparing the racemisation curves of the two proteins, and therefore he used this method to compare samples of gliadin, and to compare samples of glutenin obtained from a strong and a weak wheat.

The two samples of flour were milled from No. 1, Northern Manitoba, and from English grown wheat respectively. The method used for the isolation of the proteins was in its essentials the same as that described by Osborne[15]. He first examined the specific rotations of 2 per cent. solutions of the gliadin samples in 70 per cent. alcohol and found that the specific rotation of the gliadin from the Manitoba wheat was $- 93 \cdot 60°$, and that from the English wheat $- 93 \cdot 78°$, showing practically no difference.

The racemisation curves of the two samples were then determined, using 2 per cent. solutions in sodium hydroxide solutions of $N/2$ and $N/4$ strengths, but in each case the corresponding curves were identical,

thus indicating no differences in the structure of the gliadin samples obtained from the Manitoba and English wheats.

The specific rotations of the glutenin samples, using 5 per cent. solutions in $N/25$ caustic soda, were then determined. For the glutenin from the Manitoba wheat the specific rotation was $-99\cdot5°$, while that from the English wheat had a specific rotation of $-78\cdot8°$. Similar differences were obtained by comparing the racemisation curves of 2 per cent. solutions in $N/2$ caustic soda.

On further purification of the glutenin samples the same results were obtained, and Woodman therefore interpreted his results as demonstrating the non-identity of the glutenins from strong and weak flour. He suggested that the strong wheat synthesised one type of glutenin, and the weak wheat another, whilst wheats of intermediate strength might contain varying proportions of the two glutenins.

Gortner and Sharp[16] arrived at the same conclusion that it is the glutenin that is the controlling factor in strength by a different method. They measured the viscosity of flour in water suspensions, using a MacMichael viscosimeter. They found that all acids increased the viscosity to a maximum at a hydrogen ion concentration equal to a pH of $3\cdot0$. The viscosity of water suspensions of strong and weak flours could best be compared at this hydrogen ion concentration, the stronger flours giving suspensions in water with the higher viscosities. By washing the flour with large quantities of distilled water they found that a large proportion of the gliadin was washed out, but that very little of the glutenin was removed. A suspension of the remaining glutenin and starch had a higher viscosity in the presence of acids than before the gliadin was removed, from which they inferred that glutenin is the protein mainly responsible for the increase in imbibition when flour-in-water suspensions are acted upon by acids, and thus is responsible for strength in wheat.

A position was thus reached when further work was needed to establish the importance of the glutenin, or alcohol insoluble fraction of the gluten, in the problem of the strength of wheat flour. The following investigations were therefore carried out with this end in view. Gliadin and glutenin were extracted from various samples of flour and examined on the lines laid down by Woodman. Altogether six different flours were examined, one milled from a Rivet wheat, one from a Durum wheat, and four from English grown Fife. Woodman's results were substantiated in that it has been found that the racemisation curves of

the gliadin samples from the various wheat flours are the same, while the racemisation curves of the glutenin samples differ. It has also been found that the specific rotations of the glutenin samples vary according to the strength of the flour, the glutenin from the stronger wheat having the higher rotation.

The question then arose as to whether the glutenin from each wheat was a single protein different from those of stronger and weaker wheats, or whether it consisted of more than one protein. Did the various glutenin samples contain mixtures of say two proteins, one associated with strength in flour and the other with weakness, the strength of any particular flour depending on the proportions in which these two proteins were mixed? Experiments were therefore made to see whether the glutenin from any one flour could be resolved into different fractions, with the result that it has been found that glutenin is not a single protein, but consists of at least two fractions differing in their optical rotation. The fraction having the higher optical rotation was in predominance, which would be expected, as the fractions were obtained from the glutenin from a Fife wheat, and, as stated above, glutenin obtained from a strong wheat has a higher optical rotation than that from a weak flour. A strong wheat would therefore be expected to contain a greater percentage of the fraction with the high optical rotation.

METHOD OF EXTRACTION OF THE PROTEINS.

The method of isolation of the gliadin and glutenin from the samples of flour was somewhat different from that employed by previous workers, the differences in technique making the process much shorter. The flour was first kneaded in muslin bags under a stream of running water until most of the starch had been washed out and the gluten left behind as a rubber-like mass. The gluten was then thoroughly washed under a large number of changes of distilled water, the washing being greatly helped by mincing the gluten up finely so as to expose each time a large surface to be washed. The gluten thus obtained was found to contain approximately half its weight of water, and so strong alcohol was added in sufficient quantity to give with this water alcohol of 70 per cent. strength. The gluten was finely minced so that extraction by the alcohol was facilitated. The gluten after being extracted several times with 70 per cent. alcohol so as to remove the larger part of the gliadin, was squeezed to free it from as much alcohol as possible, and was then stirred into ·2 per cent. potassium hydroxide solution. After stirring and leaving for some time a thick colloidal solution was obtained from which the

protein was precipitated in a finely divided state on the addition of the calculated quantity of hydrochloric acid of normal strength to just neutralise the caustic potash. The protein was allowed to settle, the supernatant liquid syphoned off, and the remaining liquid separated by filtering through large filters. The finely divided protein was then stirred into 70 per cent. alcohol which easily extracted a large percentage of the remaining gliadin. This process of solution in alkali, precipitation with acid and extraction with alcohol was repeated a few times until no further gliadin could be extracted. This method of extracting all the gliadin was found to be much quicker than the method of exhaustive extraction with 70 per cent. alcohol without finely dividing up the protein by means of alkali. It was found that the gliadin was unaffected by contact with the alkali, samples obtained by the two methods having the same optical rotation.

The gliadin free residue was extracted three times with ether to remove fat after which it was stirred into ·2 per cent. caustic potash, and the solution filtered. The liquid came through water-clear on the first filtration, and thus much time was saved as previous workers have always remarked as to the great difficulty they experienced in obtaining water-clear solutions of glutenin in alkali.

The clear alkaline solution of glutenin was then neutralised with normal hydrochloric acid until a point was reached when the flocculation of the protein took place. The glutenin was allowed to settle, the supernatant liquid syphoned off and the remainder removed by centrifuging. The glutenin was well washed with 70 per cent. alcohol, finally being dried with absolute alcohol and ether, the product being a fine white powder.

The alcoholic solution of gliadin was evaporated under reduced pressure to remove the alcohol, and the concentrated solution of gliadin was poured into distilled water containing 10 grams of sodium chloride per litre. The separated gliadin was washed with distilled water and dissolved in 70 per cent. alcohol. The alcoholic solution was filtered clear, concentrated by evaporation under reduced pressure and again poured into 1 per cent. sodium chloride solution. The gliadin was redissolved in 70 per cent. alcohol, the solution concentrated and poured into absolute alcohol. The separated gliadin was dissolved in 70 per cent. alcohol, the solution concentrated and the gliadin precipitated by pouring into a mixture of absolute alcohol and ether. The gliadin was finally washed with absolute alcohol and then ether, the product being a fine white powder.

EXAMINATION OF THE GLIADIN SAMPLES.

One gram of the sample was placed in a small flask, 10 c.c. of 70 per cent. alcohol were added in which the gliadin dissolved. 25 c.c. of normal caustic soda solution were then gradually added, giving the flask, with the contained gliadin solution, a circular shaking movement so that each drop of alkali was quickly mixed with the alcoholic solution so as to prevent precipitation of the gliadin. Finally 15 c.c. of distilled water were similarly added, and the flask placed in an incubator kept at 37° C.

(1) Cambridge grown Fife

(2) Saxmundham grown Fife

(3) Durum

(4) Rivet
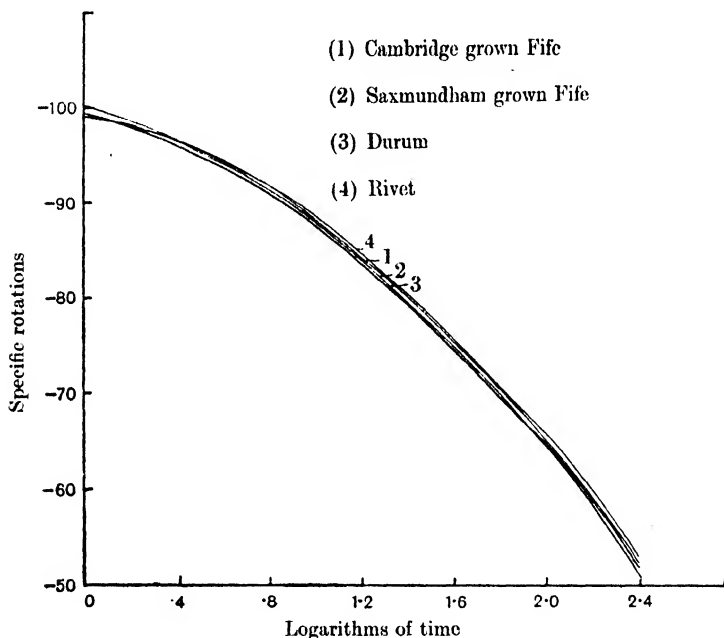
Diagram 1. Racemisation curves of gliadin samples.

At the end of one hour, the flask was taken from the incubator and cooled rapidly under the tap. The optical rotation of the solution was then determined in a decimetre polarimeter tube, using a Schmidt and Haensch instrument, and sodium light. The nitrogen was determined in two 10 c.c. samples of the solution by the Kjeldahl method, from which the concentration of gliadin in the solution was found by multiplying the nitrogen concentration by the factor 100/17·66. Thus the specific rotation of the gliadin solution could be calculated. The optical rotation was measured at stated intervals for ten days. The racemisation curves were then drawn, showing the relation of the specific rotation of

the solution to the logarithm of the time in hours, the logarithm of the time being used to obtain a more compact graph. Unfortunately the incubator was found not to be very accurate, so the experimental errors in these curves were greater than would be desired. In obtaining the racemisation curves of the glutenin samples a water thermostat was used which kept a temperature constant to within less than $\frac{1}{2}°$ C. The racemisation curves for the glutenin samples were determined after those of the gliadin samples and so are more accurate for another reason, this being that readings could be taken on the polarimeter with greater precision after practice. However, it will be seen from the curves on Diagram 1 that although they are not identical, they intertwine and vary by little, from which it is concluded that within experimental error the curves show that there is no difference in the chemical constitution of the various samples of gliadin. The gliadin obtained from the Rivet, Durum and two samples of English grown Fife were examined in this way. Thus strong and weak wheats contain the same gliadin.

EXAMINATION OF THE GLUTENIN SAMPLES.

One gram of the sample was placed in a small flask with 1 c.c. of distilled water and left so that the protein was well wetted and softened. 25 c.c. of normal caustic soda solution were then added and the flask placed in the water thermostat for 15 minutes, at the end of which time 24 c.c. of water were added and the flask left at 37° C. for another 45 minutes. At the end of the hour the flask was taken out and rapidly cooled under the tap. The optical relation of the solution was then calculated, using the factor 100/17·49. Thus the specific rotation of the glutenin was calculated. The optical rotations were measured at intervals for ten days, after which the racemisation curve was drawn, showing the relation of the specific rotation of the glutenin to the logarithm of the time in hours.

On Diagram 2 the curves will be seen for glutenin samples obtained from Rivet wheat, Durum wheat and three samples of Fife grown respectively at Royston, Cambridge and Saxmundham.

It will be seen from the curves that the glutenin samples obtained from the Royston and Cambridge grown Fifes have much higher specific rotations than those of the glutenin samples from the Rivet and Durum wheats, while the specific rotation of the glutenin sample from the Saxmundham grown Fife is intermediate, being nearer those of the glutenin from the weaker wheats.

These three samples of Fife wheat were grown from seed obtained

from Fife wheat grown successively for 21 years in England. In a report on these wheats issued by the National Association of British and Irish Millers in August 1923, this Saxmundham grown sample is stated to be inferior to the samples grown elsewhere, its strength judged by appearance being little better than well-grown English wheat.
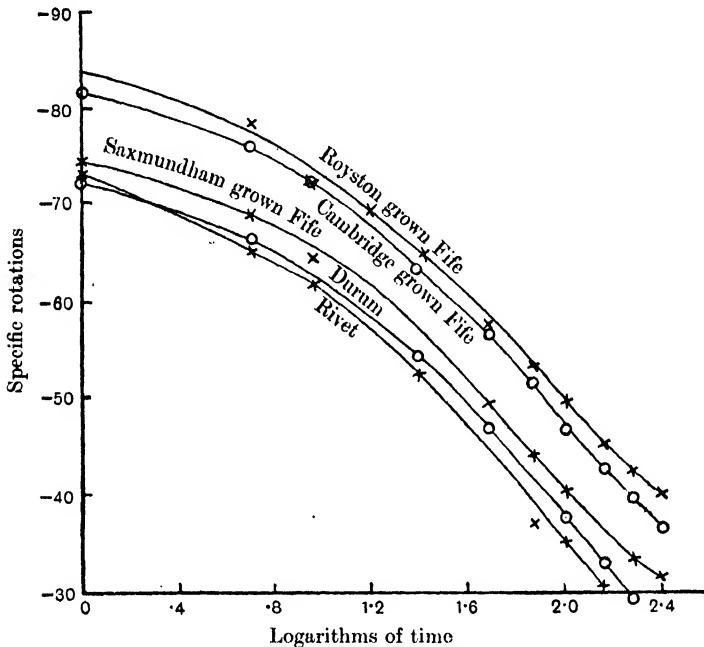

Diagram 2. Racemisation curves of glutenin samples.

A numerical expression of the relative values gives the figures:

Royston, 100.   Cambridge, 95.   Saxmundham, 75.

The volume of the loaf baked from it is also smaller than that of loaves baked from the other two. Thus it is seen that the values obtained for the specific rotation of glutenin samples obtained from various wheat flours run in the same order as the strength of the flours, the greater the strength the higher being the specific rotation of the glutenin.

EXPERIMENTS WITH THE GLUTENIN FROM KIRTON GROWN FIFE WHEAT.

Having shown that the glutenin samples obtained from wheats of various strengths differ, the question arose as to whether each sample of glutenin was a single but different protein, or whether it was a mixture of two or more proteins.

To settle this point an attempt at fractional precipitation of the glutenin from an alkaline solution was made. A clear alkaline solution of the glutenin from a sample of English grown Fife from Kirton was prepared. The solution was divided into two lots and the whole of the work outlined below carried out in duplicate, the same results being obtained in each case. Normal hydrochloric acid was then added drop by drop, stirring and leaving for a minute after the addition of each drop. The liquid gradually became more and more turbid until a point was reached when the protein separated out in a flocculent state.

The protein was allowed to settle down to the bottom of the containing vessel, when the supernatant liquid was syphoned off. This perfectly clear solution was then gradually made more acid, as before. It gradually became more and more milky in appearance until a point was reached when a second flocculation of the protein occurred, this second sample being much less than the first. This sample was allowed to settle out, the supernatant liquid syphoned off, and further acid added. No further precipitation of protein, however, occurred. The difference in the flocculation points of the two samples of glutenin corresponded to the addition of 3·8 c.c. of normal hydrochloric acid per litre of solution.

The two samples of protein were well washed with 70 per cent. alcohol and finally dried with absolute alcohol and ether.

The nitrogen content of both samples was estimated on a moisture and ash-free basis. The first sample contained 17·38 per cent. of nitrogen and the second 17·42 per cent. of nitrogen, a difference of only ·2 per cent. from each other, and a mean difference of only ·5 per cent. from Osborne's figure of 17·49 per cent. nitrogen for pure glutenin. The two samples, therefore, had the same nitrogen content, and both were pure.

The racemisation curves of 2 per cent. solutions in $N/2$ caustic soda were then determined, with the following results:

| | Specific rotations | |
Time in hours	1st sample	2nd sample
1	− 82·5	− 78·1
5	− 76·35	− 70·1
9	− 73·4	− 66·8
24	− 64·3	− 59·3
48	− 57·1	− 51·2
72	− 52·1	− 46·8
96	− 48·7	− 42·5
144	− 45·0	− 39·3
192	− 39·35	− 35·2
240	− 38·4	− 31·4

From the above data and from the corresponding curves in Diagram 3,

it will be seen that the two samples of glutenin differ appreciably in their optical rotation, thus proving that the alcohol insoluble fraction of the gluten does not consist of a single protein, but of at least two proteins which must differ in their chemical constitution.

A first fractionation was only attempted, so it is very doubtful whether complete separation was attained.
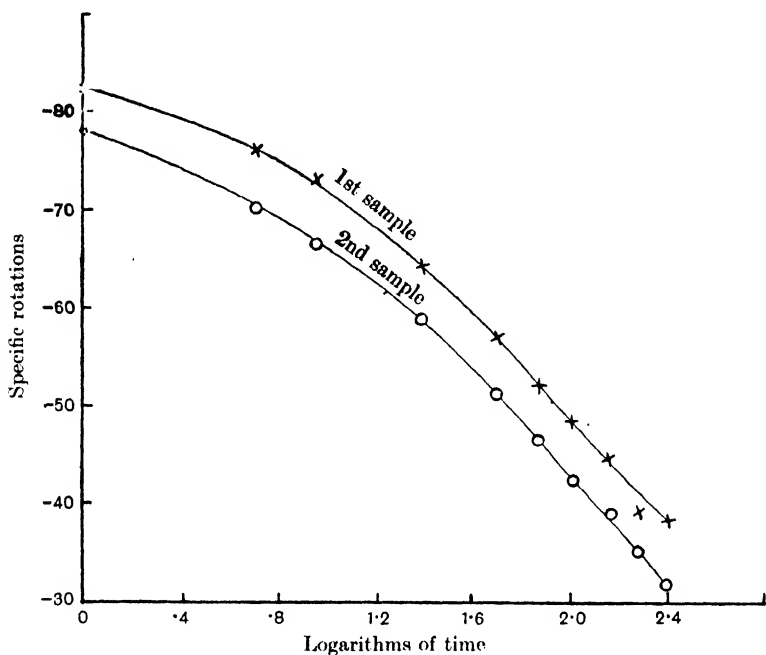

Diagram 3. Racemisation curves of glutenin samples from Kirton grown Fife Wheat.

Conclusion.

From the work outlined above it is seen that the alcohol insoluble fractions of the glutens isolated from wheat flours of different strengths vary in their specific rotations, the protein from the flours of greater strength having a greater specific rotation than the protein from flours of less strength, specific rotation and strength running parallel to one another.

It is next seen that the alcohol insoluble fraction of the gluten is not a single protein, but consists of at least two proteins, differing in their optical rotation. The flour, from which the two fractions of glutenin were obtained, was a strong one, and five times as much of the fraction with high specific rotation was obtained as of the fraction with low

specific rotation, this being in keeping with the correlation of strength and high specific rotation.

It thus seems that strength is associated with a glutenin of high specific rotation, while weakness is associated with a glutenin of low specific rotation. The work is of course incomplete and further research is needed to confirm this point. The results, however, so far obtained are important, and together with further work should throw considerable light on the problem of the strength in wheat flours.

Specific Rotations of Gliadin Samples in N/2 Caustic Soda.

Time in hours	Cambridge grown Fife	Saxmundham grown Fife	Durum	Rivet
1	− 100·1	− 99·2	− 99·0	− 99·1
5	− 92·9	− 93·5	− 92·2	− 92·5
9	− 86·9	− 88·7	− 85·9	− 88·5
24	− 79·1	− 77·0	− 77·4	− 79·4
48	− 73·7	− 70·0	− 70·2	− 73·1
72	− 68·9	− 69·7	− 67·2	− 69·1
96	− 67·1	− 65·2	− 64·3	− 65·4
170	− 60·5	− 53·2	− 55·9	− 59·7
240	− 51·5	− 50·8	− 51·8	− 53

Specific Rotations of Glutenin Samples in N/2 Caustic Soda.

Time in hours	Royston grown Fife	Cambridge grown Fife	Saxmundham grown Fife	Durum	Rivet
1	− 81·3	− 81·5	− 74·35	− 72·1	− 73·2
5	− 78·5 ·	− 76·0	− 69·1	− 66·6.	− 65·4
9	− 72·5	− 72·1	− 64·5	—	− 62·0
24	− 67·6	− 63·3	− 58·45	− 52·5	− 54·3
48	− 57·8	− 56·8	− 49·4	− 47·0	—
72	− 53·3	− 51·7	− 44·0	—	− 37·0
96	− 49·8	− 46·7	− 40·4	− 37·6	− 35·2
144	− 45·2	− 42·9	—	− 33·0	− 30·5
192	− 42·5	− 39·8	− 33·1	− 29·3	—
240	− 40·0	− 36·7	− 31·3	—	—

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STUDIES IN THE METABOLISM OF THE RUMINANT BY INDIRECT CALORIMETRY.

IV

THE INFLUENCE OF FOOD ON THE ENERGY EXCHANGE OF THE GOAT.

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(From the Rowett Institute, Bucksburn, Aberdeen.)

(With Six Text-figures.)

THE effects of food ingestion on the total energy exchange of man and of the dog have been fairly completely worked out. Lavoisier was the first to show that the oxygen absorption and carbon dioxide production were increased by ingestion of food. Rubner, in addition to confirming these observations of Lavoisier, showed that the heat given off from the body after food was equal to the heat evolved from the foodstuffs actually oxidised within the body and thus established the agreement between direct and indirect calorimetry. He showed, further, that the increase in heat production was greatest after protein food. This specific effect of protein food he designated the "specific dynamic action." The work of Rubner has been continued by, amongst others, Magnus-Levy, Benedict, and Lusk. The latter showed that the specific dynamic action of proteins is due to the special stimulating effect of certain amino-acids on the tissues and that the increased heat production after fat and carbohydrate ingestion is due to plethora of oxidisable molecules round the living body cells. Recently Wood and his school[1] have carried out investigations on the metabolism after food in pigs.

All the above investigators have worked with non-ruminant animals. There are no records in the literature of experiments of a similar nature on ruminants. Armsby and Fries[2] have, it is true, studied the metabolism of cattle after food; but their objective was chiefly the establishment of the "metabolisable energy value" of different foodstuffs rather than the physiological effects of their ingestion. Most observers in this field conducted their experiments by means of chamber methods which

are only capable of recording the metabolism in experiments of long duration. In this way transitory effects of the ingested food may have been entirely missed. The Douglas-Haldane method of indirect calorimetry is eminently adaptable for metabolic determinations at short intervals, so that it is possible by this technique to estimate most, if not all, of the temporary variations in metabolism after food.

The object of this investigation was to determine the effects of different proportions of the three classes of energy-giving foodstuffs on the energy exchange of the goat by estimating the metabolism at short and regular intervals after food. The results obtained would also, it was hoped, throw some light on the processes of digestion and assimilation and on the fermentation of food in the rumen.

EXPERIMENTAL PART.

The experiments formed four separate investigations. The first was a study of the effects of mixed food, the second of high protein food, the third of high carbohydrate food and the fourth of high fat food on the metabolism of the same animal.

The goat was pregnant when work was begun and a kid was born when the first group of experiments was about half completed. The experiments were resumed two weeks after delivery. The animal was kept in good condition by adequate daily exercise and its weight was taken daily at the same hour. In each experiment recorded the goat was standing at rest and all gross muscular movement was absent. If this were not attainable the experiment was abandoned. All the results, therefore, pertain to the minimum functional activity obtainable under the circumstances.

The Diets. In each investigation a basal ration was given daily at 5 p.m. which consisted of hay *ad lib.* In the case of the first investigation 500 grams of turnips and 200 grams of maize were given in addition as part of the evening meal. The reason for this variation was that it was decided to regard the effects of mixed food as standard, so that both the morning and evening feeds were of a mixed nature. Another meal was given daily at 9 a.m. It consisted of the foodstuffs whose effect on metabolism was the subject of investigation and was so compounded as to be of a mixed nature or to contain a high proportion of protein, of carbohydrate or of fat, according to the investigation. It was necessary also to ensure that the food was palatable and that it contained some indigestible matter. Both morning and evening feeds combined were so arranged by previous trials as to suffice for main-

tenance. The morning feeds were not varied during the course of the investigations and only slight alterations were ever necessary in the evening feeds. Administration of the appropriate dietary dated from a week before the commencement of each group of experiments, so that before any results were recorded the animal had been quite adapted to the food.

The morning or experimental meals will be subsequently referred to as the "mixed diet," the "protein diet," the "carbohydrate diet" and the "fat diet," respectively, while the evening meals will be known as "the basal rations." In Table I the caloric values of the four "basal rations" are shown.

Table I.

Diet	...	Mixed	High protein	High carbohydrate	High fat
Calories	...	1783	1017	about 1271	1271

In Table II the particulars of the diets are tabulated.

Table II.

Constituents	Total weight in grams	Digestible protein %	Digestible carbohydrate %	Digestible fat %	Calories
Mixed diet:					
Hay, Turnips, Oats	800	2·02	20·0	0·81	602·7
Protein diet:					
Dried Yeast, Oatmeal, Bran	410	25·3	39·4	2·5	1100
Carbohydrate diet:					
Maize, Rice, Barley	450	6·6	60·5	4·6	1398
Fat diet:					
Peanuts (ground), Oats	272	15·1	33·4	21·5	1066·4

The Method. Each day two metabolic determinations designated "basal" were made before 9 a.m., *i.e.* about 15 hours after administration of the evening meal. The diet to be studied was given at 9 a.m. At half-hourly intervals from 9.30 a.m. the metabolism was estimated for from 5½ to 7½ hours after eating. The other experimental conditions in both *ante*, and *post*, *cibum* experiments were exactly similar and have been described more fully in an earlier paper(3). At least ten values for each half-hourly interval after food were obtained, a number which previous experience had shown to be desirable to secure a reliable average.

Determination of Base Line. As pointed out by Krogh and Lindhard(4) it is preferable to take as the base line the average of all the basal values obtained rather than to take each daily basal value as a separate base line for that day. The former procedure was adopted for all the basal

values except those of the mixed diet. During this investigation parturition occurred and the basal metabolism was, as has been pointed out (3), a variable value. In the *ante partum* period it was increasing from day to day and in the *post partum* period decreasing from day to day. This difficulty was overcome by constructing graphs of the basal metabolism during the investigation (Fig. 1).
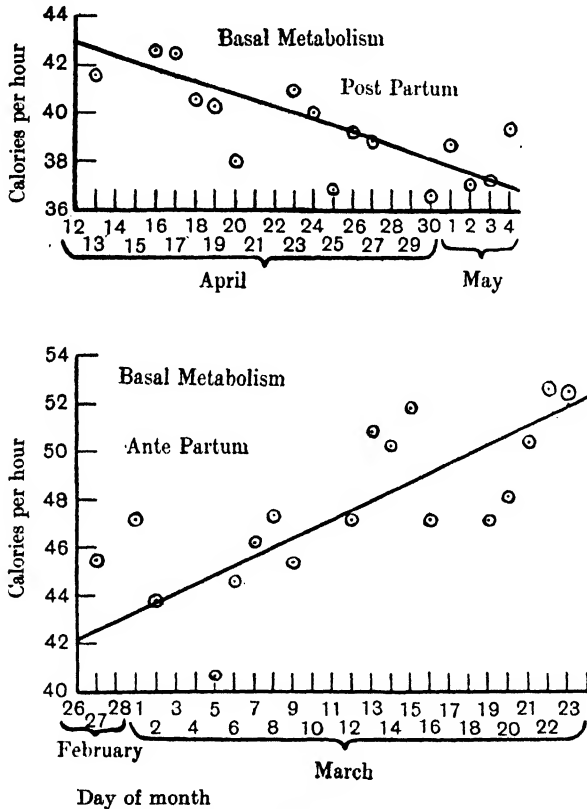

Fig. 1.

The basal values are ordinates and the days of the month abscissae. Straight lines were obtained by drawing as evenly as possible through the points. It will be seen that the average deviation of the points from the respective curves on the upper side is approximately equal to the average deviation from the same curve on the lower side. The curves, therefore, are an approximate representation of the basal metabolism during the investigation and are more reliable for comparative purposes than the day to day findings.

Compilation of Results. The results of the investigations on the protein, carbohydrate and fat diets were appropriately tabulated and then averaged. Thus there were obtained for each of these diets the average basal values of the tissue heat, the R.Q. and the fermentation heat and also average values of the same for each half-hourly interval after food. Curves were then plotted with these values as ordinates and the time after food as abscissae (Figs. 4 to 6). As an example the tissue heat results for the carbohydrate diet are shown in Table III.

Table III. *High Carbohydrate Diet. Tissue Heat in cals. per hour.*

						Time after food in hours						
Basal		½	1	1½	2	2½	3	3½	4	4½	5	5½
50·7	51·7	55·3	54·5	56·6	54·2	55·3	52·2	58·4	59·8	59·1	60·4	56·0
54·5	46·3	56·0	56·2	55·8	55·1	54·8	52·6	59·3	68·0	60·2	60·2	58·9
32·7*	38·0	52·0	53·1	50·0	51·7	52·0	59·7	63·5	61·6	62·2	57·2	60·0
60·8	64·9	49·8	48·1	48·3	51·5	52·8	62·6	63·2	67·9	54·3	59·9	63·3
52·4	53·3	51·5	67·4*	54·8	68·6	62·3	64·5	59·7	57·9	59·7	56·9	56·1
47·3	49·6	56·2	58·5	53·1	59·8	61·2	65·3	60·5	59·3	59·3	59·6	62·3
50·3	41·0	61·7	62·5	58·4	61·8	60·1	58·7	60·4	63·1	54·2	67·7*	60·2
51·7	51·3	55·1	57·5	57·4	54·6	59·3	55·5*	59·0	62·3	59·0	59·3	52·2
59·3	56·2	57·8	59·7	59·3	57·0	56·9	62·5	61·3	63·8	59·3	52·6	51·3
45·8	48·3	52·8	51·8	52·6	48·0	62·6*	62·4	56·9	53·0	55·7	55·0	59·4
54·9	51·1	55·1	53·4	—	57·8	60·1	68·6*	57·1	59·6	59·0	65·5	—
59·1	40·7	—	—	—	—	59·6	—	—	—	—	—	—
52·0	50·7											
52·3	51·2											
49·5	43·2											
51·1	47·0											
50·9	52·3											
50·9	52·3											
Av.... 50·8		54·8	55·5	54·6	55·1	58·1	60·4	59·9	61·5	58·3	58·7	58·0

* Rejection.

In the case of the mixed diet it was impracticable to average either the basal metabolism or the gross after-food metabolism owing to the effects of the pregnancy and puerperium factors. The increments were, however, obtainable by deducting the basal metabolism from the appropriate after-food metabolism. In this way the increments at each half-hourly interval after ingestion were secured and averaged as above. It is evident, however, that the average increment so obtained for, say 7½ hours after food, represents the influence of the pregnancy and puerperium factors in addition to the food factor for a period of 7½ hours. It was estimated from the curves in Fig. 1 that when the six values of the *ante partum* period are combined with the five of the *post partum* period the net average effect of the pregnancy minus the puerperium factors for 7½ hours was + ·04 calorie per hour. This value had then to be subtracted from the value at 7½ hours after food in order to obtain

the increment due to food. Since the correction decreases as the interval approaches zero time after food, and, because it is insignificant in amount, it was dispensed with.

It was possible to average the R.Q. values, both basal and after-food, in the *ante partum* period, as the pregnancy factor had apparently no significant progressive effect on the R.Q. The puerperium factor also had no significant progressive effect on the R.Q.; but as there were certain differences in the results before and after parturition they were averaged separately in the manner described under the other diets. For similar reasons the fermentation heat results were treated likewise. Curves were then plotted as above from the average values obtained (Figs. 2 and 3).

In obtaining the mean the principles laid down by Gephart, Du Bois and Lusk(5) were employed, but these were superfluous in the case of the R.Q. values, which were averaged in the ordinary way after discarding values showing gross departure from the general trend.

COMPARISON OF DIETS.

In Table IV a comparison of the characteristics and effects of the four diets has been made. The chief points of interest in the results of each investigation are here shown in a condensed form. From the foregoing it will be clear that it was impossible to calculate the percentage increments in metabolism in the case of the mixed diet. The average basal metabolism value in the table, 37·7 calories per hour, is that obtained in the last four days of the *post partum* experiments when the metabolism had become steady. The R.Q. and fermentation heat values were, however, calculable on a percentage basis; but only the *post partum* values were employed for this purpose as they were considered more desirable for comparison.

Physical Characteristics. It will be seen that the mixed diet was the heaviest and most bulky and the fat diet the least so, while the protein and carbohydrate diets were approximately equal in both respects. The protein and fat diets were equal in caloric value while the carbohydrate diet was 30 per cent. higher and the mixed diet 40 per cent. lower.

Effects on Metabolism. The animal's basal metabolism was highest on the carbohydrate diet and lowest on the mixed diet. An explanation of the former unexpectedly high figure is to be found in the fact that the animal was "in heat" during nearly the whole of this investigation. Presumably the amount of the food increment was not influenced thereby. Excluding this abnormal figure the highest basal rate is that for the

Table IV. *Comparison of Diets.*

	Physical characteristics			Metabolism						R.Q.				Fermentation heat			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Diet	Wt. in grams	Relative bulk	Caloric value	Average basal value	Maximum increment actual and percentage and time of occurrence	Average increment for 5¼ hrs. actual and percentage	Increment at ¼ hr. actual and percentage	Increment at 2¼ hrs. actual and percentage	Increment at 5¼ hrs. actual and percentage	Basal R.Q.	Maximum R.Q.	Average R.Q. for 5¼ hrs.	Average R.Q. 2¼ hrs. to 5¼ hrs. inclusive	Basal fermentation heat	Average for 5¼ hrs. and percentage increment	Increment at ¼ hr. actual and percentage	Average 3 to 5¼ hrs. inclusive and percentage increment
Mixed	800	Largest	602·7	37·7	9·4 at 2¼ hrs.	6·2	6·6	9·4	3·6	·77	·83	·78	·78	2·25	3·23-43·6	1·24- 55·1	2·34-40
Protein	410}	Equal	}1100	46·6	13·5-28·8 at 5 ”	10·8-23·2	6·8-14·6	11·6-24·9	12·5-26·8	·74	·85	·80	·79	2·99	5·24-75·2	4·99-166·9	4·37-46·1
Carbohydrate	450}		1398·1	50·8	10·7-21·1 at 4 ”	6·9-13·6	4·0- 7·9	7·3-14·4	7·2-14·2	·81	·86	·83	·82	3·75	5·37-43·2	2·03- 54·1	5·36-42·9
Fat	272	Smallest	1066·4	41·8	10·4-24·9 at 3¼ ”	7·8-18·7	4·2-10·0	8·8-21·0	8·4-20·1	·78	·88	·83	·81	2·37	3·22-35·9	2·18- 91·9	2·72-14·7

protein diet. Thus the "secondary effect" of high protein feeding of Rubner is demonstrated in the ruminant as has been often shown by many observers in non-ruminant animals (Lusk[6], Cathcart and Orr[7] and others).

The maximum increment observed was greatest after the protein diet, 13·5 calories per hour or 28·8 per cent. above basal. It was next greatest after the fat diet. It is unfortunate that, owing to the absence of the percentage figures, the mixed diet results could not strictly be compared with the others. It will be noticed, that the maximum increment of the mixed diet occurred at $2\frac{1}{2}$ hours or an hour earlier than after the fat diet whose maximum increment appeared the next earliest.

The figures in the seventh column again bring out the pronounced effect of protein on heat production. The average increment for $5\frac{1}{2}$ hours is greatest for this diet and next greatest for the fat diet, which, as observed, was fairly rich in protein.

The increment after the first half-hour was greatest for the protein diet, next greatest for the mixed diet and least for the carbohydrate diet. Similar remarks apply to the increment after $2\frac{1}{2}$ hours.

The rapid falling off in the effect of the mixed diet, and of the carbohydrate diet to a much less extent, is brought out in the tenth column.

Effects on the R.Q. The basal R.Q. is highest on the carbohydrate diet. This confirms the findings of Benedict and Higgins[8] who showed that a carbohydrate meal raises the R.Q. in the succeeding *post absorptive* state.

It is noteworthy that the highest R.Q. obtained, ·88, was after the fat diet and that those after the protein and carbohydrate diets were almost equal.

The figures in the thirteenth column show that the average R.Q. for $5\frac{1}{2}$ hours was equal in the cases of the fat and carbohydrate diets.

The average R.Q. for $2\frac{1}{2}$ hours to $5\frac{1}{2}$ hours inclusive is highest for the carbohydrate diet but only ·01 higher than that of the fat diet, the next highest. This period, as will be explained later, corresponds with the time of maximum absorption of the mixed, protein and carbohydrate diets. The corresponding period for the fat diet was from the first to the fourth hour. The average R.Q. of this period was ·84 or ·02 higher than for the carbohydrate diet.

Effects on Fermentation. The basal fermentation heat is highest for the carbohydrate, and next highest for the protein, diet. Evidently the quality of the food has an influence on the excretion of fermentation gas 24 hours after ingestion.

The average fermentation heat for $5\frac{1}{2}$ hours is greatest for the carbohydrate diet and somewhat less for the protein diet, but the percentage increment over basal is much the greatest for the latter. The figures in columns 15 and 16 together with those in the last column, which represent the fermentation heat due to the diets as distinct from the basal ration, indicate that the concentrated diets induced greater fermentative activity in the basal rations and themselves fermented more thoroughly, than the mixed diet. Starchy foods also seem to ferment more thoroughly than other concentrates. This opinion confirms that of Armsby and Fries(2) and of Kühn(9) who found that the more carbohydrate a ration contained the more extensive was the fermentation.

From the seventeenth column it is evident that the protein diet caused a much more rapid expulsion of accumulated gas than the other diets.

<div style="text-align:center">DISCUSSION OF RESULTS.</div>

The following points of interest will be apparent after inspection of the curves and the foregoing comparison table.
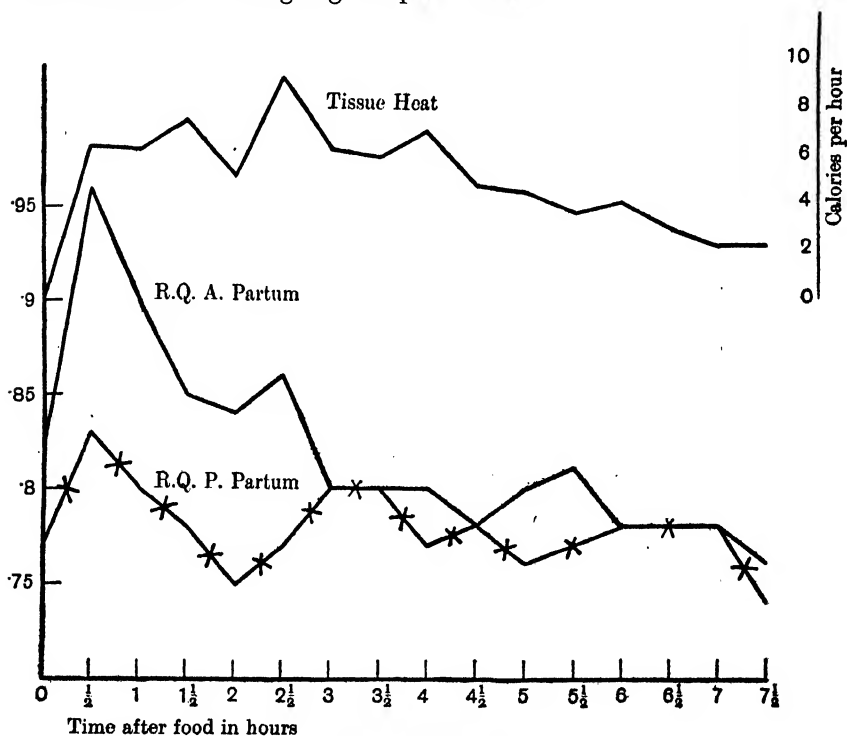
Fig. 2. Mixed diet.
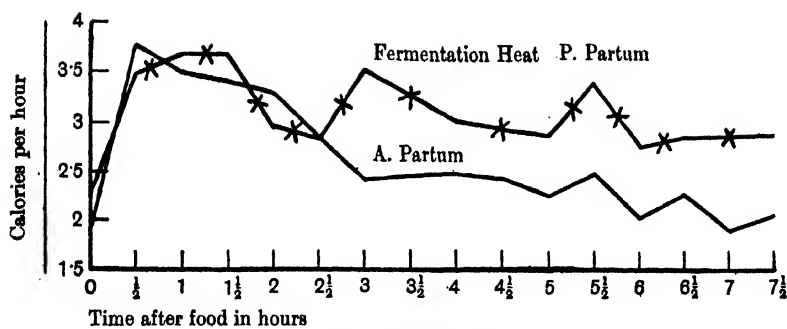
Fig. 3. Mixed diet.
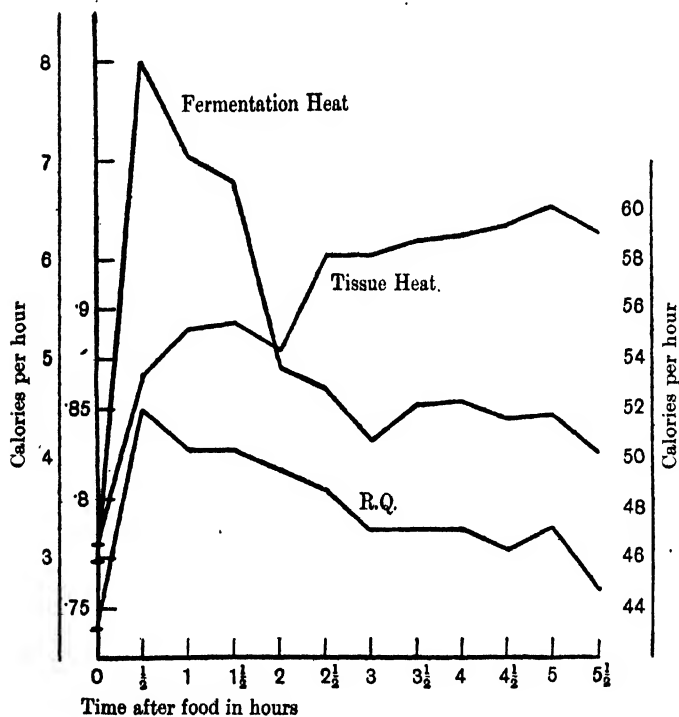
Fig. 4. Protein diet.
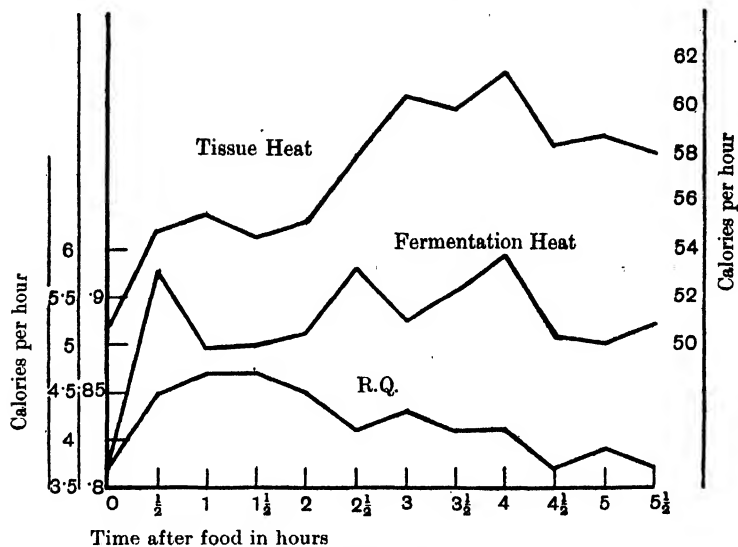
Fig. 5. Carbohydrate diet.
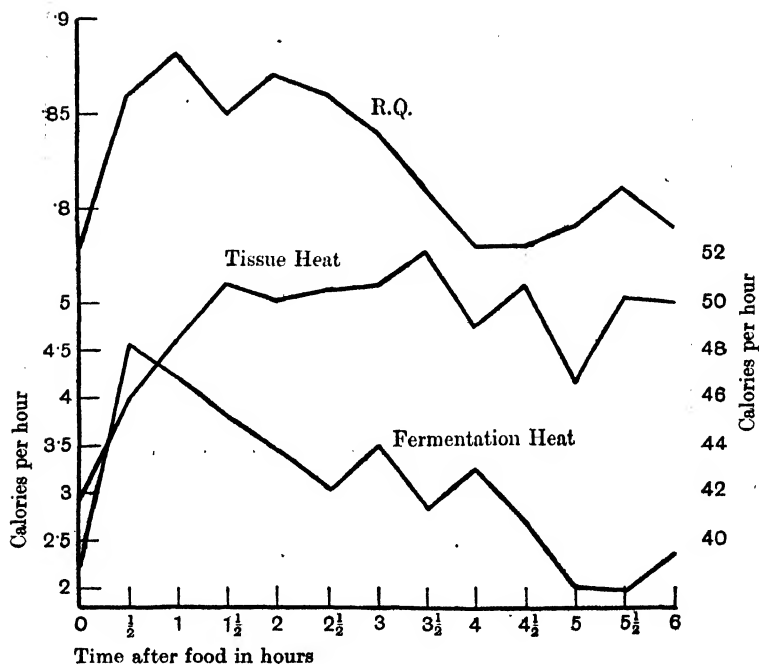
Fig. 6. Fat diet.

I

The metabolism curves of the mixed, protein, and carbohydrate diets are strikingly similar for the first $2\frac{1}{2}$ hours. After the increase at the first half-hour the metabolism remains almost at the same level for the next $1\frac{1}{2}$ hours, then all three curves show a big rise at $2\frac{1}{2}$ hours. These facts are suggestive of a fundamental difference between the metabolic response to food in ruminants and that in omnivora and carnivora. In the latter the *post cibum* metabolism curves that have been described show, generally, a gradual and uninterrupted increase to a maximum and then a gradual decline. The maximum increment, in addition, often appears soon after ingestion. Thus, Lusk[10] obtained a maximum increment of 88 per cent. in the dog between two and three hours after a meat meal, Benedict and Carpenter[11] one of 21 per cent. in man two to three hours after a banana-mixture meal, and Magnus-Levy[12] one of 33 per cent. in man an hour after a meal of bread. It is true the maximum increment appeared at $2\frac{1}{2}$ hours after the mixed diet. But this departure from the type of the curves of the protein and carbohydrate diets is obviously due to the low caloric value of the mixed diet which was unable to maintain the metabolism at a high level for long. Furthermore, the available calories of the mixed diet were contained principally in the roughage, and the necessity for extensive fermentation prevented the early entrance of soluble products into the metabolic field in considerable amount. It was not so in the case of the protein and carbohydrate diets whose maximum increments appeared at the fourth and fifth hours respectively. The pronounced increase at $2\frac{1}{2}$ hours is a definite landmark in the *post cibum* metabolism curves of these three diets. The possible causes for the increased metabolism up to this time will now be discussed.

1. The delayed effects of mastication cannot be held responsible, for the animal was never observed eating when the first sample of expired air was about to be drawn off.

2. The theory that the "work of digestion" occasions any increase in heat production is no longer tenable for the following reasons.

If the theory is sound the heavier and bulkier the meal the greater should be the increase in metabolism. In the present investigation this was not found to be so. The protein and carbohydrate diets were equal in bulk and the latter a little heavier than the former but considerably higher in calorie content. Nevertheless, at no point in the metabolism curves did the carbohydrate diet produce as great an increment as the

protein diet. Again, the mixed diet was three times as heavy as, and several times more bulky than, the fat diet. Yet the latter produced a greater maximum and a greater average increment in metabolism than the former. Furthermore, Benedict and Emmes[13] have shown that sodium sulphate and agar-agar, and Benedict and Carpenter[14] that large quantities of warm water have no influence on metabolism. These agencies increase the peristaltic activity of the alimentary canal. Lusk[15], too, demonstrated that extract of meat and urea and sodium chloride were without influence on metabolism. These substances stimulate the secretory and absorptive functions of the alimentary canal. There does not appear, therefore, to be any substantial basis for belief in the stimulating effect on metabolism of the so-called "work of digestion."

3. It may be claimed that the initial increase in metabolism is due to stimulation of cell activity by the absorbed digestion products of the diets. If this were so it is curious that the metabolism should have remained practically stationary for two hours after ingestion, and also that changes in the R.Q. corresponding to the nature of the diet were not observed. As shown below, the changes in the R.Q. up to $2\frac{1}{2}$ hours have little if any relation to the oxidation of the diets ingested; so that the operation of some other stimulant of metabolism is extremely probable.

4. For reasons given below it is probable that the increase in metabolism for the first two hours after the three diets under consideration is due to nervous or hormone stimulation of cell activity as the immediate effect of the ingested food.

(a) The feeling of warmth and well-being after ingestion of a good meal when hungry comes on almost immediately, a phenomenon that, owing to its early onset, does not appear easy of explanation by the specific dynamic action of the food.

(b) Dmitrenko[16] inflated the dog's stomach with a balloon and observed an increase in pulse rate and blood pressure as a result.

(c) Maydell[17] isolated a "secretin" from the dog's stomach and, on injecting it into the blood, dilatation of blood vessels amongst other results ensued.

All these facts appear interrelated and seem to add weight to the above contention. Furthermore, the fact that the initial increment was greatest after the protein diet and next greatest after the mixed diet is of importance, in that the protein diet was one rich in gastric secretogogues, and the mixed diet one that caused distension of stomach and rumen.

II

The behaviour of the R.Q. after all four diets is noteworthy. In the first place the R.Q. up to three hours after the first three diets and up to 1½ hours after the fat diet will be considered and then the remaining sections of the curves.

1. All four curves rise sharply at the first half-hour and then fall again more or less sharply. These changes bear little apparent relation to the oxidation of the ingested diets. Thus the R.Q. was ·85 at half-an-hour after the protein diet and the same at half-an-hour after the carbohydrate diet. The former fell, it is true, to ·83 at the next interval and to ·82 at the second hour; but the highest point reached by the latter was ·86 at one and 1½ hours, and it fell again to ·85 at the second hour. Therefore, some other factor was most probably responsible for these changes in the R.Q.

Dodds[18] and Bennett and Dodds[19] have shown that, after food, the concentration of alveolar CO_2 rises above the fasting level during gastric secretion and falls during pancreatic secretion, and that the rise and fall are best marked after foods rich in gastric secretogogues. It is reasonable to assume that a rise in alveolar CO_2 should produce a rise in the R.Q. and the converse a fall. It will be noticed that an acceleration in the fall of the R.Q. occurs at 3 hours after the protein diet, at 2½ hours after the carbohydrate diet and at 1½ hours after the fat diet, i.e. at times corresponding to the commencement of the secondary increase in metabolism after the protein and carbohydrate diets respectively, and to a significant increase in metabolism after the fat diet. When the first considerable rush of digestion products into the blood occurred duodenal digestion must have been proceeding apace. Therefore, the above-noted accelerations in the fall of the R.Q. occurred about the time of the commencement of duodenal digestion of the respective diets. The absence of an acceleration in the fall of the R.Q. after the mixed diet can be explained by its high roughage content which was held back in the rumen to ferment. It is evident, therefore, that the rise and fall in the R.Q. may be attributed chiefly to the effects of the digestive cycle, and that the acceleration in the fall coincident with a considerable increase in the metabolic rate, marks approximately the time when duodenal digestion was at its height. It seems, therefore, that in the case of the first three diets the entry of the first considerable amount of food into the intestine commenced at about 2½ hours, and in the case of the fat diet at about an hour, after ingestion.

2. For the remaining portion of each curve the low level of the R.Q. is remarkable. As already explained the average R.Q. during the hours of maximum oxidation was highest after the fat diet. The average R.Q. after the carbohydrate diet was ·82, which is too low for the oxidation of glucose. Two explanations are offered.

(*a*) When cellulose ferments, organic acids of the average composition of butyric acid are formed (Markoff[20]). It has been seen that all foods, and particularly concentrates, ferment in the rumen, producing most probably, acids like butyric and lactic and their salts. The combustion R.Q. of butyric acid is ·80; but if an alkaline salt is oxidised and a bicarbonate formed the R.Q. is only ·60:—

$$C_3H_7COOK + 5O_2 = KHCO_3 + 3CO_2 + 3H_2O.$$

Lusk[21] points out that the similar oxidation of sodium lactate gives a R.Q. of ·67. If alcohols are formed from the fermenting food, as does not seem improbable considering that they have been discovered recently in the culture media of an intestinal organism (Khouvine[22]), they also would give a low R.Q. As will be shown below, fermentation gas commenced to be evolved from the food from 2 to 3 hours after ingestion. The soluble products that were then set free by fermentation would on oxidation give rise to a low R.Q. After the carbohydrate diet some glucose set free from starch that escaped fermentation was most probably being oxidised from $2\frac{1}{2}$ hours onwards. Consequently the R.Q. is higher than during the corresponding period after the protein or mixed diet.

(*b*) Lusk[23] suggests that the cause of the increased heat after carbohydrate ingestion is the plethora of acetaldehyde molecules round the body cells. It is just possible that in the ruminant aldehyde is formed from the fermentation products as well as from glucose. It follows from the work of Hill[24] and his school that the working muscle uses up lactic acid. It is extremely probable that the combustion occurs in stages with the formation of intermediaries. If lactic acid is heated acetaldehyde and formic acid are formed:

$$CH_3CHOHCOOH \rightarrow CH_3CHO + HCOOH.$$
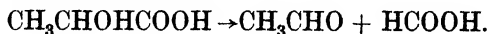
As the organic acids set free in the rumen are most probably absorbed in the form of salts, the above change, if it occurred in the animal, would result in the formation of a formate. The interaction of the formate with absorbed butyrate would give another molecule of aldehyde:

$$\begin{matrix} C_3H_7COOK \\ + \\ HCOOK \end{matrix} \longrightarrow C_3H_7CHO + K_2CO_3.$$
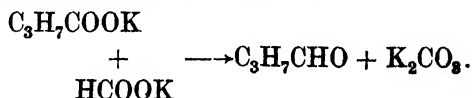
The combined R.Q. of equimolecular amounts of acetic and butyric aldehyde is ·76, so that if these assumptions are correct they offer an explanation for the low R.Q. and the increased metabolism during absorption of carbohydrates.

III

The results of the fat diet can be seen to differ in many important particulars from those of the other diets. The divergencies may be explained as follows.

The fat diet compared with the others was of very small bulk and, on account of the ground peanuts, of porridgy consistency. For these reasons it began to enter the intestine in considerable amount between a half and 1½ hours after ingestion as is apparent from the rising metabolism and the dip in the R.Q. at 1½ hours. That the diet was being oxidised during this period is proved by the high R.Q. at the first hour, ·88. This is an increase of ·10 over basal, which is too great to be attributed entirely to the effect of gastric secretion since the corresponding increment after the bigger and more albuminous protein diet was ·11. The R.Q. of ·88, therefore, was partially the result of oxidation of the carbohydrate of the diet. Fermentation could not have gone as far as the production of bodies with a low R.Q. within an hour, so that the carbohydrate metabolite oxidised must have been glucose split off from unfermented starch.

IV

All the curves of the fermentation heat are roughly similar in general outline. The sharp initial rise is undoubtedly due to mechanical stimulation of the musculature of the rumen and stomach by the ingested food. Since the rise is sharpest after the protein diet and next sharpest after the fat diet, which was next richest in nitrogen, the intensity of the peristaltic activity is related to the nitrogen content of the diet. That the accumulated gas was excreted more gradually and slowly after the mixed and carbohydrate diets is proved by the plateau in the one curve and the slow fall in the other after the initial rise.

From 2½ to 3 hours after the respective diets the fall in the fermentation heat curve is succeeded either by a check in the fall or by a rise. This indicates that gas commenced to be evolved from the fermenting food at this time. Owing to the activity of the stomach at this stage the gas could not accumulate but was excreted. The succeeding peaks on the curves indicate temporary exacerbations in the activity of the stomach as it is expelled with masses of food into the duodenum. It is

easy to imagine that, when the stomach expels a mass of food, some gas is eructated from the rumen at the same time. In this connection it is significant that there is a peak on the metabolism and fermentation heat curves at 4 hours after the carbohydrate diet, and two on the fermentation heat curve of the fat diet, at the third and fourth hours, which are followed by peaks at $3\frac{1}{2}$ and $4\frac{1}{2}$ hours on the metabolism curve. The half-hour's delay in the metabolic response in the case of the fat diet is most probably due to the time required for the passage of absorbed fat into the blood via the lacteals and thoracic duct. The low R.Q. from the fourth to the fifth hour seems to strengthen this view. The comparative evenness of the metabolism curve of the protein diet from $2\frac{1}{2}$ hours and of the fermentation heat curve of the same from three hours appear to indicate that protein food causes the stomach to expel its contents in a small steady stream. Incidentally, the steady and even rise in the metabolism curve of the protein diet contrasted with the irregularity and more rapid falling off in the carbohydrate curve show that protein metabolites have a more lasting and cumulative effect, whereas carbohydrate metabolites have a transient effect, on heat production.

It will be evident that the rate of excretion of fermentation gas is not a measure of the rate of fermentation until $2\frac{1}{2}$ to 3 hours after food, and that thenceforward it is only an approximate measure; for, as observed, the expulsion of masses of food from the stomach causes gas to be excreted more rapidly than it is formed.

It will be noticed that in the first investigation the *ante partum* R.Q. is on the whole on a higher level than the *post partum* R.Q., and that the *ante partum* fermentation heat rate is lower than the *post partum* rate. These findings substantiate the views expressed in an earlier paper[3].

CONCLUSIONS.

1. The changes in the rate of metabolism of the ruminant due to food are different from those of omnivora and carnivora.

2. After ingestion of a meal of 400 grams or more the metabolism of the goat increases after half-an-hour to a level which remains fairly steady for two hours. It is suggested that this initial increment is due to stimulation of metabolism by a nervous or hormone mechanism activated by the food.

3. A secondary increase in metabolism commences at $2\frac{1}{2}$ hours after a meal of sufficient size. This signifies the commencing absorption in

significant amount of the ingested food. After a meal of small bulk and weight the initial increment only lasts for about half-an-hour. In this case the secondary increment is superimposed on the initial increment an hour after ingestion, thus indicating considerable oxidations of the ingested meal at this time.

4. As in omnivora and carnivora: (1) protein food has the most pronounced and most lasting effect, (2) fatty foods have a lesser but a lasting effect, (3) carbohydrates have a comparatively small and transitory effect, on the heat production of the goat.

5. The rise and the subsequent fall that occur in the R.Q. soon after food ingestion are associated with the secretion of the digestive juices. These changes are most marked after protein food.

6. Fermentation sets free soluble bodies which have a low R.Q. on oxidation. Concentrates ferment more extensively than mixed rations and carbohydrates more than other concentrates. A small meal of concentrates loses less available energy by fermentation than a large meal of concentrates. Gas begins to appear in the fermenting food about three hours after ingestion. Up till this time gas formed from the previous meal is for the greater part being excreted. This excretion of gas is the result of mechanical stimulation of the rumen and stomach by the ingested food. From about the third hour the rate of excretion of gas is only an approximate guide to the fermentation heat rate; the former increases out of proportion to the latter when the stomach expels food masses into the intestine.

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STUDIES IN THE METABOLISM OF THE RUMINANT BY INDIRECT CALORIMETRY.

V

THE COURSE OF METABOLISM AFTER FOOD IN THE GOAT.

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(With one Figure in the text.)

THE nearest approach obtainable to the true basal metabolism of an animal is "the metabolism corresponding to the minimum functional activity" (Krogh(1)). The two conditions essential for the determination of this metabolism, commonly, but erroneously termed "basal," are complete muscular rest and the *post absorptive* state. In all animals the first condition is unattainable, unless recourse is had to drugs; but by training and an invariable routine a state of muscular activity that is, for all practical purposes standard, can be secured. In omnivora and carnivora the *post absorptive* state is reached 12 hours after the last food, but there are no published records to show when it is reached in ruminants. The absence of such experimental records in the literature provided the necessity for this investigation, the chief objective of which was the determination of the time of the onset of the *post absorptive* state in the goat.

THE EXPERIMENTS.

The animal, the same as had been employed in the earlier studies of this series, was in excellent condition when the work was begun. A liberal mixed ration of 2526 calories was given daily in a pre-period of three weeks during which time the basal metabolism was estimated almost daily.

During the experimental period of 84 hours no food was given, but water was allowed *ad lib.* The animal displayed no anxiety for food and appeared to be quite comfortable. The environment was warm and quiet. Secretion of milk diminished but slightly. Cudding was observed for the last time at 41 hours after food.

On 30. x. 23 at 5 p.m. the usual evening feed was given, and at 6 p.m. the remains of the meal, about 350 grams of straw, were removed. Metabolic determinations were begun at 6 a.m. the following day, *i.e.*

12 hours after the last food and continued throughout the experimental period. Three samples of expired air of 10 to 12 minutes duration were collected at each interval except the last three at which, owing to diminished ventilation, duplicate samples of 15 minutes duration were obtained.

Table I.

Time after last food in hours	Tissue heat in calories per hour	Average	Fermentation heat in calories per hour	Average	R.Q.	Average
12	50·9		3·71		·842	
,,	49·2	49·6	3·70	3·83	·751*	·84
,,	48·7		4·09		·830	
18	42·2		1·67		·786	
,,	44·3	43·1	2·13	1·63	·791	·80
,,	42·9		1·08		·811	
24	39·7		1·09		·767	
,,	39·4	39·3	·97	·92	·743	·76
,,	38·7		·70		·761	
30	36·4		1·15		·722	
,,	37·6	36·8	·76	·98	·781	·74
,,	36·3		1·02		·715	
36	35·1		·68		·725	
,,	38·0	38·0	·49	·57	·758	·72
,,	41·0		·55		·693	
42	40·9		·41		·666	
,,	39·4	39·4	·44	·42	·677	·67
,,	38·0		·44		·665	
48	34·1		·26		·701	
,,	36·8	37·1	·37	·39	·666	·67
,,	40·3		·53		·649	
54	34·9		·37		·659	
,,	36·6	36·6	·39	·37	·693	·68
,,	38·4		·34		·686	
60	33·8		·29		·690	
,,	35·2	34·6	·50	·36	·674	·68
,,	34·9		·29		·680	
66	39·1		·36		·645*	
,,	38·0	38·2*	·25	·30	·672	·66
,,	37·4		1·15*		·659	
72	34·3	35·8	·77	·77*	·667	·66
,,	37·3		·77		·656	
78	28·0*	—	·20	·28	·740*	·67
,,	37·0*		·36		·673	
84	34·1	32·3	·18	·25	·642	·67
,,	30·6		·33		·690	

Average pre-period, tissue heat   45·7 cals. per hour.
,,   ,,   fermentation heat   2·55   ,,
,,   ,,   R.Q.   ·78   ,,
* Rejection.

The mean values for the experiments of the pre-period were found and then the three (or two) values for each six-hourly interval were tabulated and averaged (see table). Unfortunately a complete series of

results was not obtained. A few had to be discarded on account of the behaviour of the animal or mishaps in the technique. Curves were plotted, the time after food being the abscissae and the average values for the tissue heat, R.Q., fermentation heat, respiration rate and weight the ordinates. The appropriate values obtained in the experiments of the pre-period were taken as the starting point of each curve.
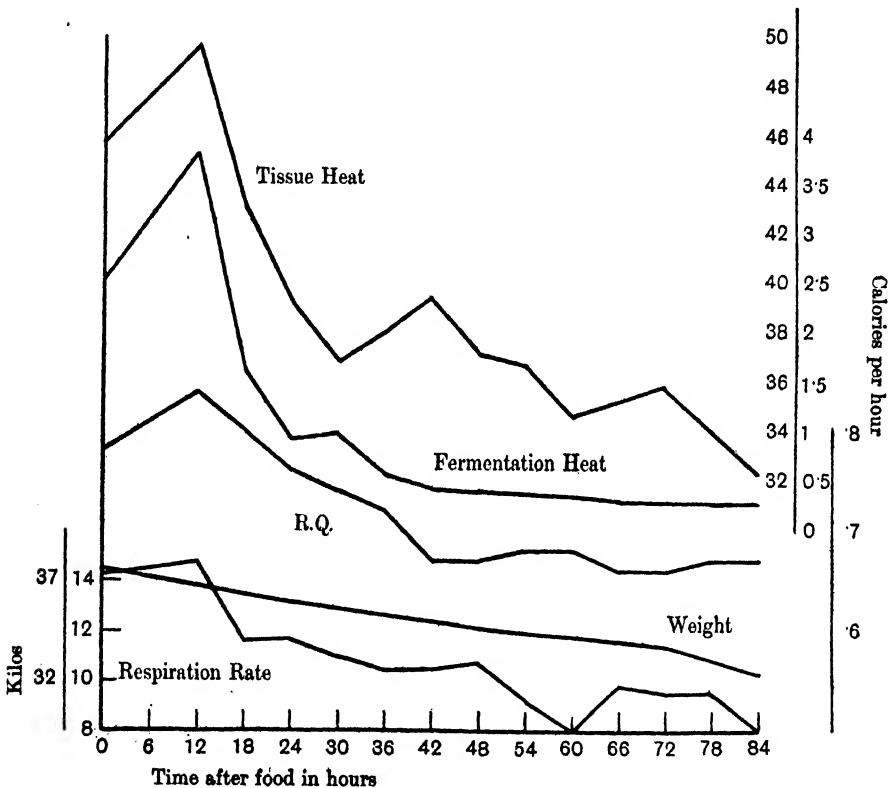

Fig. 1.

THE RESULTS.

After consideration of the results in the table and of the graphs the following points will become evident.

1. There are peaks at 12 hours after food on the curves of the tissue heat, R.Q., and fermentation heat. The starting points on these curves are the average values in the pre-period which relate to a time 15 hours after the last food. The values at the 12th hour, therefore, were obtained three hours earlier after ingestion of the same evening meal minus

350 grams of straw. The increase in the R.Q. from ·78 to ·84, signifying increased oxidation of carbohydrate, would seem to indicate that the increase in metabolism is attributable to the three hours priority in the 12th hour experiments. Similarly, the increase in the fermentation heat rate might be accounted for. It may be, however, that the removal of the 350 grams of straw compensated for the three hours priority, so that some other factor may have been responsible. Kunde[2] observed an increase in metabolism above "basal" in the early days of fasting which disappeared if the stomach were filled with inert indigestible matter. Kunde attributed the increased metabolism to reflex excitation of muscle tonus by hypertonic contractions of the empty stomach. Since the rumen very probably contained less bulk at the 12th hour than when the experiments of the pre-period were carried out, it is possible for the increase in metabolism and in the fermentation heat to be partially accounted for by hypertonicity of the rumen and stomach.

2. The metabolic rate after falling rapidly from the 12th to the 30th hour rises through the 36th hour to a peak at the 42nd and then falls rapidly again. This sudden increase in metabolism was undoubtedly due to oxidation of the remains of the last food, since cudding was observed for the last time at the 41st hour. As has been pointed out in a former paper [3] the oxidation of the products of fermentation give a low R.Q. so that the sudden drop in the R.Q. at the 42nd hour appears conclusive evidence that the rumen and stomach were completely evacuated at this time. The *post absorptive* state, therefore, was reached, and true fasting commenced, between 42 and 48 hours after food.

This finding has an important bearing on some of the earlier studies in this series. All the earlier "basal" determinations were generally carried out 15 hours after the last food. This investigation shows that some food must have been present in the rumen when these determinations were made. The state of digestive activity of the animal, like the state of muscular activity was, however, kept fairly constant by unvarying diets and fixed times of feeding. Obviously, the standard metabolism obtained under these conditions is considerably higher than that obtainable in the *post absorptive* state as the above results show. Thus the pre-period value, which is "basal" in the sense attached to this term in earlier investigations, is 8·6 calories per hour or 23 per cent. higher than the value in the *post absorptive* state, *i.e.* at 48 hours. But as the standard, or "basal," metabolism was constant throughout each investigation the comparative value of the results was not impaired.

In an earlier paper [3] it is suggested that the initial increase in

metabolism after food ingestion is attributable to nervous or hormone stimulation of cell activity. The above findings, however, raise the question as to the exact rôle played by the food that was already in the rumen when the various foodstuffs were administered. Did it alone produce the initial increment in metabolism, or, having become mixed with the incoming meal, did it remain in the rumen for a time facilitating the fermentation of the newly ingested food ultimately to contribute towards the specific dynamic action of the latter? The second conception of the course of events, which involves the operation of some such factor as hormone stimulation, is the most probable. The other is unlikely for the following reasons.

(a) The initial increment in metabolism varied according to the nature of the food just ingested, being greatest after protein food.

(b) There was insufficient time between the administration and the ingestion of the food for the residue in the rumen to be expelled into the duodenum in order to be absorbed; for thus only could the latter, as a separate entity, influence metabolism.

(c) The caloric value of the contents of the rumen cannot have been high enough to produce such a large increase in metabolism which amounted to 14·6 per cent. in the case of the protein diet.

3. The R.Q. from the commencement of the *post absorptive* state until the 84th hour remained at an average level of ·67 and the excretion of fermentation gases during the same time decreased very slowly and had not ceased at the 84th hour. In other words when the animal was drawing upon the reserves within its own body for sustenance the R.Q. was below that of fat oxidation and fermentation gases continued to be excreted for over 36 hours after their source had been cut off. These results open up two questions: (a) whether any gas was absorbed from the rumen and excreted via the lungs and (b) whether the fermentation ratio of Krogh and Schmit-Jensen(4), on which the adaptation of this technique to the goat is based, is too high.

(a) Fries, discussing the uncertainty of the R.Q. in ruminants(5), considers it very probable that some gas is absorbed from the rumen and excreted via the lungs. He cites Zuntz and Lehman as having found CH_4 in the expired air of the horse in which fermentation of food occurs chiefly in the colon. There are, however, no records of experiments that deal with the absorption of fermentation gas in ruminants. In this investigation the fermentation heat rate at the 84th hour, 0·25 calorie per hour, is equivalent to the excretion of 56·6 c.c. of gas in a collecting period of 15 minutes. This amount is too considerable to be entirely

accounted for by absorption. If, in the fasting animal, fermentation gases were absorbed to an appreciable extent, the more soluble gas, CO_2, would disappear more rapidly than CH_4; so that the actual ratio, in which these gases were being excreted, would be higher in the early hours of fasting and lower in the later hours than the ratio employed in the calculation, viz. $\dfrac{CO_2}{CH_4} = 2 \cdot 6$. Consequently, in the calculation, which has already been explained in detail (Orr and Magee(6)), the volume of CO_2 attributed to fermentation would be too small in the early hours of fasting and too big in the later hours. There would thus be attributed to tissue metabolism too much CO_2 in the former case and too little in the latter, so that in the early hours of fasting the calculated R.Q. would be too high and in the later hours too low. But, since the R.Q. in this investigation remained almost steady from the commencement of true fasting, it is extremely improbable that any considerable amount of gas was absorbed into the blood stream and excreted by the lungs.

(b) If the fermentation ratio of Krogh and Schmit-Jensen, $\dfrac{CO_2}{CH_4} = 2 \cdot 6$, were too high the R.Q. calculated by means of it would be too low. Thus the low R.Q. from the 42nd hour onwards could be explained. The ratio was based on the results of many carefully planned experiments carried out *in vitro* to study the fermentation of the rumen contents of slaughtered cattle. The cattle had previously been fed on roughage and it is just possible that had they been fed on concentrates in addition a lower ratio might have been found. The ratio of Mollgaard and Andersen(7), $\dfrac{CO_2}{CH_4} = 2 \cdot 8$, is a little higher. It was obtained by experiments on the living cow fed on roughage, concentrates and roots. If one employed this ratio instead of $2 \cdot 6$ in the calculation the R.Q. would be lowered by about $\cdot 003$; so that it would not be appreciably altered. The low R.Q. therefore, seems to indicate that both ratios are probably a little too high but that Krogh's is a nearer approximation to the truth.

Another possible explanation is that decomposition of the contents of the large intestine during the fast gave rise to oxidisable products with a low R.Q. This seems the most probable interpretation; but owing to the lack of experimental data that would throw light on this question a definite conclusion cannot be arrived at.

SUMMARY.

The *post absorptive* state commences in the ruminant between 42 and 48 hours after the last food. If food is ingested before this time it becomes mixed with that already in the rumen and its decomposition by fermentation is thereby accelerated. The metabolism at 15 hours after the last food is about 23 per cent. above that in the *post absorptive* state. For about 36 hours after the commencement of the *post absorptive* state the R.Q. remains below that of fat oxidation and fermentation gases still continue to be excreted. Reasons are suggested by way of explanation for these phenomena in the causation of which absorption of gas from the rumen does not appear to play any appreciable part.

NOTE. The authors regret that it is not at present possible to continue further this series of investigations. Many of the views put forward in this and the preceding study are more in the nature of suggestions for further research than definite conclusions, and it is believed therefore, that research along the lines indicated would be well repaid. The authors hope that it may be possible at some future time to resume these studies.

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THE FORM OF MECHANICAL COMPOSITION CURVES OF SOILS, CLAYS, AND OTHER GRANULAR SUBSTANCES.

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(With Two Text-figures.)

In an earlier paper by the present writer[1], describing a new method of mechanical analysis, allusion was made to the use of curves for expressing the mechanical composition of soils or clays. The curves, examples of which were given, were made to show the relationship between summation percentages and logarithms of settling velocity in water. Since the publication of the paper in question, the writer has examined a large number of soils and other materials by the new method and drawn their curves. A consideration of these curves has revealed certain regularities which will be discussed in the present paper.

MATERIALS USED AND METHOD OF WORKING.

It is of course impossible to reproduce more than a selection from the large number of curves which have been obtained. Typical examples are however given in the diagrams. The experimental points were obtained by the writer's method[1] which, as pointed out in the earlier paper, enables a wide range of particle size to be examined with comparatively little trouble. It may be mentioned that in the case of data obtained for long settling periods, the suspensions were kept in stoppered bottles in dark cupboards at reasonably constant temperatures in order to avoid disturbances by radiation and convection currents.

The following are the particulars of the soils and other materials whose compositions are given in the diagrams.

Diagram I. (*a*) London Clay.
(*b*) Kaolin.
(*c*) Powdered Slate.
(*d*) Powdered Felspar.
(*e*) Powdered Diorite.

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[1] Since the publication of the former paper it has appeared that the period of shaking recommended, 2 to 4 hours is not always long enough. A period of 12 to 18 hours is probably safer if complete dispersion is to be effected.

Diagram II.   (a) Carboniferous Limestone Clay, Bangor.
               (b) Subsoil Clay, Harpenden, Herts.
               (c) Alluvial Subsoil Clay, Denbighshire.
               (d) Lias Soil, Glamorganshire.
               (e) Boulder Clay Subsoil, Carnarvonshire.

Diagram III.   (a) Palaeozoic Silt Loam Subsoil, Denbighshire.
               (b)   Ditto   Ditto   another sample.
               (c) Boulder Clay Subsoil, Anglesey.
               (d) Glacial Loam, Bangor.
               (e) Subsoil, Anglesey Medium Loam.

Diagram IV.   (a) Average Comp. Palaeozoic Silt Loam Soils.
               (b)   Ditto   Ditto   Subsoils.
               (c) Average Comp. Anglesey Medium Loam Soils.
               (d)   Ditto   Ditto   Subsoils.

It should be explained that the data represented in the diagrams refer to ignited weights. In ordinary soils and clays, therefore, the curves can never reach 100 per cent. The deficit represents hygroscopic moisture and less on ignition. Since any point on the curves gives the percentage of material having a settling velocity less than that of the corresponding $\log v$ of the abscissae, the percentage of material in any range can be found from the difference between the corresponding ordinates. For example the percentage of "fine silt" is given by the difference between the ordinate corresponding to $\log v = \bar{4}\cdot0000$ and the ordinate corresponding to $\log v = \bar{2}\cdot0000$.

The curves are shown in the four diagrams of Fig. 1. We shall now proceed to discuss certain regularities which are brought to light from a consideration of these and other curves.

SMOOTHNESS OF MECHANICAL COMPOSITION CURVES.

In the first place, the tendency of the experimental points to lie on smooth curves may be remarked. That this is not a consequence of the small number of observations made is supported by the results obtained where experimental points are taken fairly close together over the range. It is seen that comparatively little "smoothing" is necessary to obtain very satisfactory curves. This is the case with a large number of curves in addition to those shown in the diagrams. It would seem therefore that if the results of a mechanical analysis be set out in the form of curves of this kind, interpolation can be safely used to obtain inter-mediate points. A comparatively small number of determinations are

thus required to characterise the mechanical composition of a soil or clay. Greater detail is of course required for materials of mixed origin as for example, certain alluvial deposits.
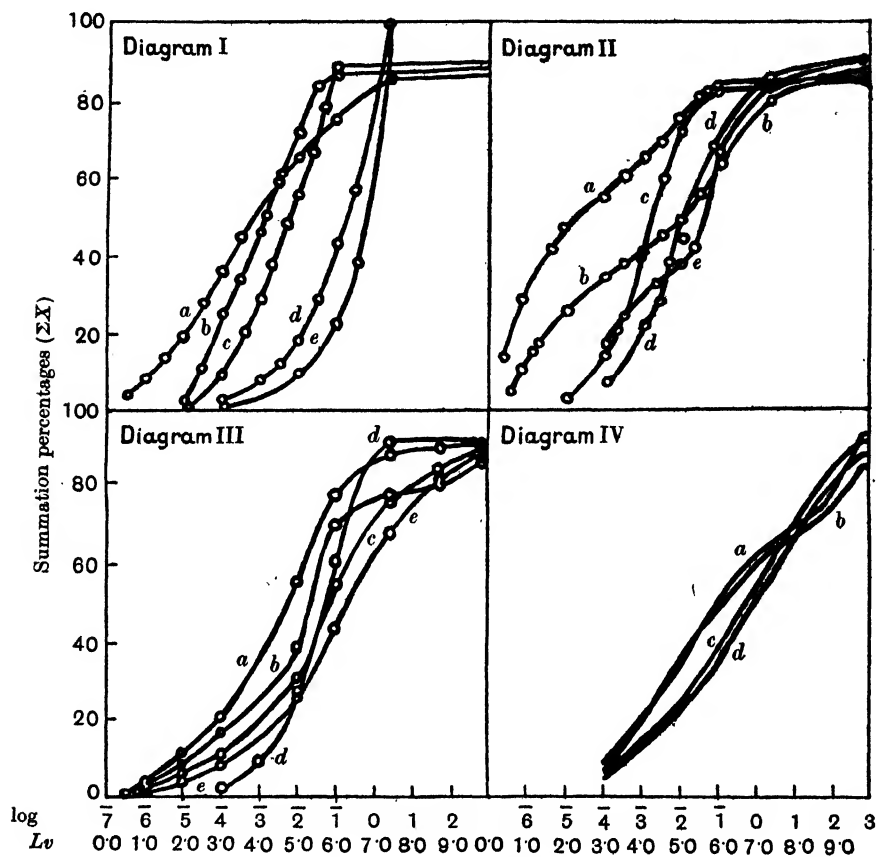

Fig. 1. Mechanical composition curves.

A recognition of these regularities will be helpful in view of the large number of different systems of grading used in different countries[1]. Since the separation of fractions is generally based on settling velocities in water, where these are known, conversion from one scale to another by interpolation is possible. For example in a recent investigation of two soils from Holland, kindly supplied by Dr D. J. Hissink for another purpose, mechanical analysis was first carried out using the English

[1] This has already been mentioned by C. L. Whittles, *Journ. Agric. Sci.* 1922, **12**, 166.

conventional scale. From the curves obtained, the values on the "Atterberg" scale were obtained by interpolation and were found to agree to ·5 per cent. with the values obtained experimentally on that scale.

FORM OF CURVES.

The actual form of the curves obtained is worthy of notice. Considering the curves for ordinary soils and clays, it is found that they
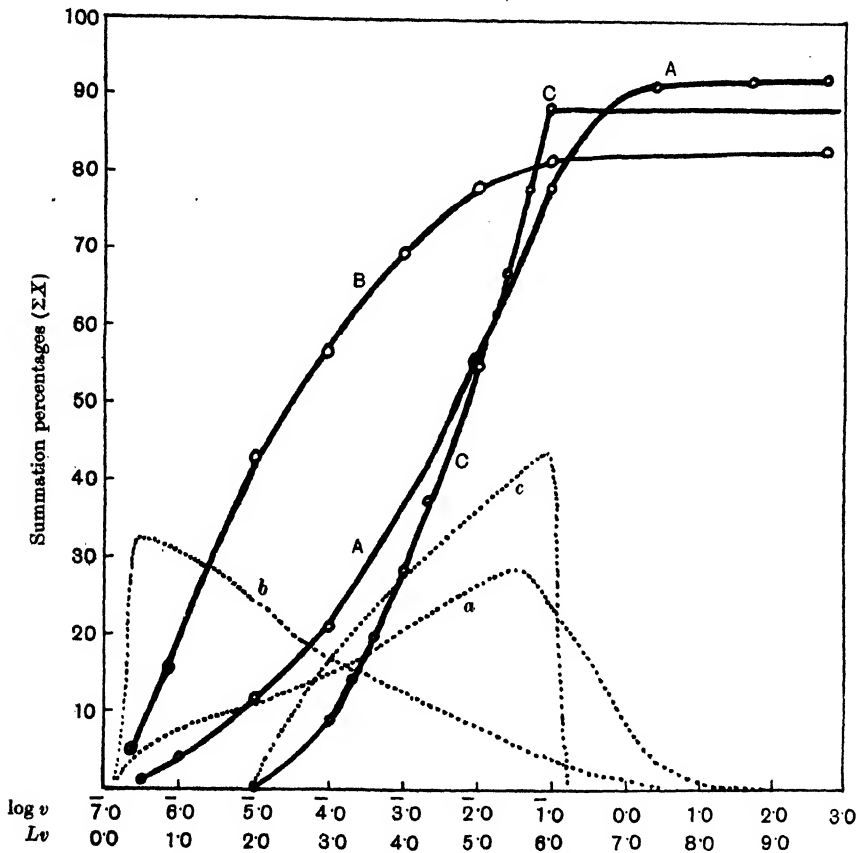

Fig. 2. Principal types of mechanical composition curves.

show one general type with two sub-types. For illustration of these types, three curves are shown in Fig. 2. The principal type, to which the great majority of soils belong, is exemplified by curve A. It is a sigmoid with the point of inflection corresponding to the steepest part

of the curve. This portion represents the fraction present in greatest amount. A curve showing the relationship between $d\Sigma X/d \log v$ and $\log v$, obtained graphically from the summation curve, gives an idea of the relative frequency of the material in different parts of the range. The fraction corresponding to the steepest part of the summation curve and to the maximum on the differential curve may be termed the "*modal fraction*" of the material. In the case of the soil represented by the summation curve A and by the differential curve a, the modal fraction is at $\log v = \bar{2} \cdot 5000$ which in the English scale is slightly above the lower limit of the fine silt. The modal fraction can of course be readily found from inspection of the summation curve without drawing the differential curve and may be a useful single value for classification purposes.

It is desirable in any system of grading to be devised to use equal logarithmic intervals. This is the case in the Atterberg scale where the successive limits are $2 - 0 \cdot 2 - 0 \cdot 02 - 0 \cdot 002$ mm. The approximate position of the modal fraction is then shown directly. The writer would prefer $\log v = \bar{5} \cdot 0 - \bar{3} \cdot 0 - \bar{1} \cdot 0 - 1 \cdot 0$ as successive limits.

Two variants of the main type were encountered. In the first place certain very heavy clays, as for example that characterised by curves B and b, appear to have the lower portion of the sigmoid suppressed, so that the modal fraction falls at the lower limit. This was found to be the case for two Carboniferous clays and for a clay subsoil from Trinidad. It would appear that in the case of other clays, this type of curve would be obtained but for a small admixture of coarser material. Such a case s given by curve II b.

In the case of certain materials which have been reduced to powder by mechanical grinding, as for example slate powder, represented by curves C and c, the modal fraction appears to be at the upper limit. It is also to be noticed that the range of particle size is not so extended as in the case of soils and clays.

COMPOSITE CURVES.

Besides the curves belonging to the types above described, there are some curves, as for example II b and II e, which seem to represent composite materials. These curves have been encountered in mixed drifts, and alluvia in which successive strata have been mixed together in the sample.

Certain cases in which material of uniform origin give composite curves may be specially considered. There occur in North Wales considerable tracts of soil in which large proportions of partially disinte-

grated rock are present. Such soils are "immature" soils in the early stages of development from Palaeozoic rocks. They are soils in which the decomposition has been mainly mechanical and this is reflected in the high proportions of fine gravel, particularly in the subsoils. The curves for such soils show a second steep portion near the upper end, suggesting a second modal fraction. But as the upper limit is artificial on account of the removal of material coarser than 3 mm., it is probable that if all the material were included, the complete curve would consist of two successive sigmoid portions. In Diagram IV, the average composition of soils and subsoils of two of the principal soil types of North Wales are shown. Both the Silt Loams show the steep portion at the upper end, and there is a suggestion of it also in the subsoil curve for the Anglesey Medium Loam.

It is possible that for such soils the upper limit for soil might be more appropriately taken at 1 mm., thereby eliminating the coarser material which consists of gravel and rock fragments. This does not imply that the coarser material is to be neglected. It may, however, be convenient for some purposes to study the two classes of material separately.

<div align="center">LOWER LIMIT OF PARTICLE SIZE.</div>

The form of the curves shown in the present paper suggests that the lower limit in the case of ordinary soils and clays is in the region of $\log v = \overline{7}\cdot0000$ and there is no indication in any of the curves examined of a limit below this. On the other hand, in the case of kaolin and the mechanically disintegrated powders, the lower limit seems to be in the region of $\log v = \overline{5}\cdot0000$. The conclusion reached as to the lower limit for ordinary soils and clays is in fair agreement with the conclusions reached by Whitney[2] and implied in a recent paper by Thomas[3].

The case of kaolin merits special discussion. This material contains a considerable proportion, about 24 per cent., of clay on the conventional English scale. On the Atterberg scale it would show about 35 per cent. of clay. It is not however a typical clay and is devoid of plasticity. The lower limit for this material seems to lie, as mentioned above, at about $\log v = \overline{5}\cdot0000$. May it not therefore be desirable to take the limit for clay at this point in order to show up more clearly the difference between plastic substances and materials which, although in a fine state of division, lack the characteristic properties of clays?

SUGGESTED METHOD OF EXPRESSING PARTICLE SIZE.

We shall probably err in assuming that the small settling velocities at the lower end of our curves can be translated into terms of particle size by the application of the Stokes formula. As was shown in the former paper, the presence of a gel coating would materially affect the settling velocity of the smallest particles. For this reason, the writer would again urge the desirability of distinguishing fractions simply on the basis of their settling velocities, thereby avoiding a hazardous assumption as to the validity of Stokes' law over the whole particle range.

The use of the logarithm of the settling velocity has appeared to be a convenient solution. It must be admitted, however, that there is a disadvantage in the use of negative logarithms. Since the lower limit of particle size appears to be at $\log v = \bar{7}\cdot0000$, it is tentatively suggested that this point should be taken as zero, in other words that $\log (v \times 10^7)$ should be substituted for $\log v$. This suggests, on the analogy of the use of pH as a measure of hydrogen-ion concentration, that Lv might be used as a symbol to indicate settling velocity and, by inference, particle size. This is illustrated by the following equivalents:

	$\log v$	Lv
Lower limit	$\bar{7}\cdot0000$	$0\cdot0000$
Suggested clay	$\bar{5}\cdot0000$	$2\cdot0000$
English clay	$\bar{4}\cdot0000$	$3\cdot0000$
Atterberg clay	$\bar{4}\cdot5506$	$3\cdot5506$
English fine silt	$\bar{2}\cdot0000$	$5\cdot0000$
English silt	$\bar{1}\cdot0000$	$6\cdot0000$

There is of course the objection that the property which it is sought to measure by mechanical analysis is particle size and that in spite of using settling velocity as an equivalent, there will always be the tendency to calculate back to terms of particle size. This is admitted to be justifiable over a large part of the range of particle size. But the uncertainty as to the validity of Stokes' formula occurs in that part of the range which is of the greatest importance from the point of view of actual physical properties. At the lower limit, particle size is as much an abstraction as logarithm of settling velocity. With regard to the use of the term "equivalent diameter" or its logarithm, it appears to the writer that there can be no objection to such a term as long as it is realised that it is an abstraction. On the whole, however, it is probably better to avoid the use of any expression which tends to obscure an uncertainty.

SUMMARY.

1. The mechanical analysis of a number of soils, clays, and other granular materials has been carried out by the writer's method and the results have been set out in the form of curves showing the relationship between summation percentages and logarithm of settling velocity.

2. The curves obtained are smooth. This suggests that all the necessary information as to the mechanical composition of a material can be obtained by using a relatively small number of experimental points and drawing the appropriate curve. Results obtained on one scale can readily be transferred to another scale by interpolation.

3. The most common type of curve is a sigmoid. The steepest portion of the curve represents the fraction present in greatest frequency. This has been termed by the writer the *modal fraction*. Two sub-types were found. In the case of certain heavy clays, the modal fraction appears to be at the lower end of the curve, whilst in the case of materials mechanically disintegrated the modal fraction is at the upper limit of particle size.

4. Composite curves are obtained in certain cases. They may represent mixtures of different materials, or soils in the earlier stages of formation from rock.

5. The lower limit of particle size appears to be represented by $\log v = \bar{7}\cdot0000$ in the case of normal soils and clays. In the case of certain finely divided but non-plastic materials, the lower limit appears to be in the region of $\bar{5}\cdot0000$. It is suggested that a better picture of the properties of soils would be obtained by taking the limit for clay at this point instead of at $\log v = \bar{4}\cdot0000$.

6. The use of the logarithm of settling velocity as a measure of particle size is discussed and it is suggested that the use of negative logarithms might be avoided by using $\log (v \times 10^7)$, for which the symbol Lv is proposed.

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ON THE MEASUREMENT OF HYDRION CONCEN-TRATION IN SOME DAIRY PRODUCTS BY MEANS OF BIILMANN'S QUINHYDRONE ELECTRODE.

By VERA LESTER.

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(With Three Text-figures.)

IT has hitherto been rather a difficult matter to measure the hydrion concentration in milk, cream, buttermilk and whey.

By the colorimetric method it is necessary to dialyse the electrolytes of the fluid through a parchment- or collodion-membrane and then measure the reaction in the clear dialysate. The parchment-dialysis requires a long time and if it is not done at low temperature and with strict precautions to prevent bacterial infection, the fluid will be altered, frequently forming acids. The dialysis through collodion sacs no doubt takes place rather quickly (equilibrium in about 30 minutes), but it takes a long time to prepare the sacs, and they are not very strong. The dialysis technic is however subject to a systematic error—although a small one—caused by the Donnan-effect, and moreover the colorimetric estimations cannot always be made with sufficient exactitude.

When analysing by the platinated hydrogen electrode a Höber-Hasselbalch electrode vessel with fixed hydrogene-atmosphere has to be used and it has to be shaken because of the carbon dioxide contained in the fluid. This method has many sources of error and is rather troublesome. In some cases it will take several hours to obtain a constant potential, and as the reaction of the milk is changed by being

kept, it will from this reason already be desirable to search for a quick, easy and exact method and so much more as serial researches may prove of interest to the dairy technical as well as to the physician.

Consequently I tried to apply Biilmann's quinhydrone electrode. As to the theory of the quinhydrone electrode I must refer to Biilmann's publications [1].

<div align="center">EXPERIMENTAL.</div>

I have determined the reaction in full milk, cream, whey and butter-milk. As the measurement in buttermilk requires a special procedure, this will be mentioned separately.

<div align="center">(1) Measurement in full milk, cream and whey.</div>

Into a small glass beaker is poured 10–20 c.c. of the fluid which is to be examined + about 5 ctgr. quinhydrone; after stirring, three smooth gold electrodes are put into the fluid together with the siphon-tube from a KCl-bridge, which forms the connecting link with the standard electrode.
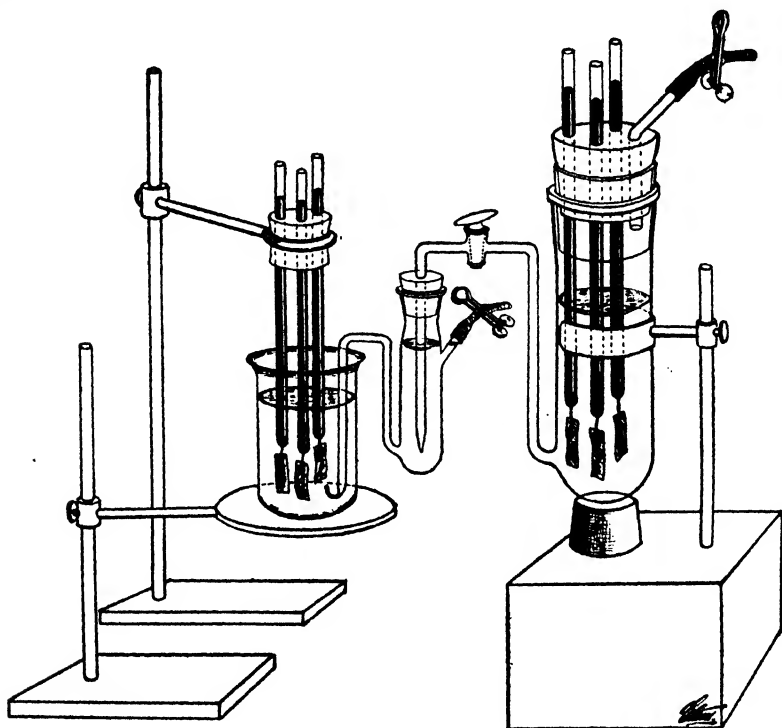

<div align="center">Fig. 1.</div>

The arrangement is shown in Fig. 1. The glass beaker on the left contains the examined fluid of which the hydrion concentration is to be measured. The vessel on the right is a Quinhydrone Standard Electrode (2) as proposed by Biilmann and Veibel; three smooth platinum or gold electrodes in a saturated solution of quinhydrone in $n/100$ HCl which at the same time contains 9/100 gram-mol KCl per litre; the pH in this fluid is about 2·03[1]; an ordinary calomel-electrode vessel filled with 3·5 c.c. KCl is placed air-tight on the siphon tube of the standard electrode; the siphon-tube of this vessel is drawn out in a thick capillary tube which is bent sharply and cut off as shown in Fig. 1. This impedes the diffusion of the heavy KCl-solution into the fluid to be measured.

Instead of the KCl-bridge here described an inverted U-tube filled with the ordinary solution of KCl stiffened with agar may also be used; the one end of the tube is then placed in the quinhydrone electrode, and the other in a small glass beaker with a solution of 3·5n KCl in which the siphon-tube of the standard electrode has also been placed. This method was originally described by Michaelis and used for determining the hydrion concentration in samples of soil by H. R. Christensen and Tovborg Jensen (3).

The potential becomes constant in the course of less than five minutes, and pH is calculated according to the formula

$$pH = 2{\cdot}03 + \frac{\pi \text{ volt}}{0{\cdot}0577}$$

by 18° C., π indicating the found potential of the standard electrode in comparison with the electrode, which has been measured.

Ten samples of full milk, ten samples of cream, and ten samples of whey have been examined; each sample was measured with the quinhydrone electrode as well as with the platinated hydrogen electrode in the Höber-Hasselbalch electrode vessel with three electrodes.

The results of the measurements are shown in the Tables I, II and III. It will be seen that the pH values according to the two methods generally differ less than 0·05; but that in each series is a single test where the difference reaches 0·10. However it is very difficult to decide whether it is the platinated hydrogen electrode or the quinhydrone electrode which gives the correct, or the most correct, value. But the difference is in any case of no practical importance.

As the cream is rich in fat and only contains few electrolytes it is difficult to obtain a good conductivity in the Höber-Hasselbalch electrode,

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[1] According to yet unpublished measurements which Prof. N. Bjerrum has been kind enough to communicate to me, the exact value is 2·029.

where one is obliged to measure with closed glasscock. This makes the measurement inexact. By the quinhydrone electrode this inconvenience does not exist. *Whey* quickly becomes very acid when kept standing, up to 0·1 pH unit per hour; consequently the quick quinhydrone electrode is much to be preferred to the slow platinated hydrogen electrode.

Table I. *Measurements in full milk.*

	Quinhydrone electrode pH	Hydrogen electrode pH	Difference
(a)	6·67	6·76	− 0·09
(b)	6·66	6·76	− 0·10
(c)	6·67	6·74	− 0·07
(d)	6·67	6·70	− 0·03
(e)	6·62	6·67	− 0·05
(f)	6·62	6·69	− 0·07
(g)	6·61	6·66	− 0·05
(h)	6·62	6·65	− 0·03
(i)	6·65	6·65	0
(j)	6·65	6·60	+ 0·05

Table II. *Measurements in cream.*

	Quinhydrone electrode pH	Hydrogen electrode pH	Difference
(a)	6·49	6·55	− 0·06
(b)	6·54	6·58	− 0·04
(c)	6·54	6·54	0
(d)	6·54	6·56	− 0·02
(e)	6·59	6·62	− 0·03
(f)	6·54	6·63	− 0·09
(g)	6·58	6·55	+ 0·03
(h)	6·56	6·56	0
(i)	6·48	6·41	+ 0·07
(j)	6·62	6·66	− 0·04

Table III. *Measurements in whey.*

	Quinhydrone electrode pH	Hydrogen electrode pH	Difference
(a)	5·77	5·73	+ 0·04
(b)	5·98	6·06	− 0·08
(c)	6·02	6·10	− 0·08
(d)	6·01	6·05	− 0·04
(e)	6·01	6·03	− 0·02
(f)	5·76	5·78	− 0·02
(g)	5·65	5·66	− 0·01
(h)	5·53	5·49	+ 0·04
(i)	5·72	5·62	+ 0·10
(j)	5·75	5·69	+ 0·06

(2) *Measurements in buttermilk.*

The caseine in buttermilk is precipitated in larger or smaller clots, which will stick to the smooth electrodes making it impossible to obtain

a constant potential without taking special precautions. Consequently I tried to obtain a clear fluid through filtration, but this procedure did not prove satisfactory whichever filter I used, as in the best case the filtration took so long time that the reaction had become considerably changed.
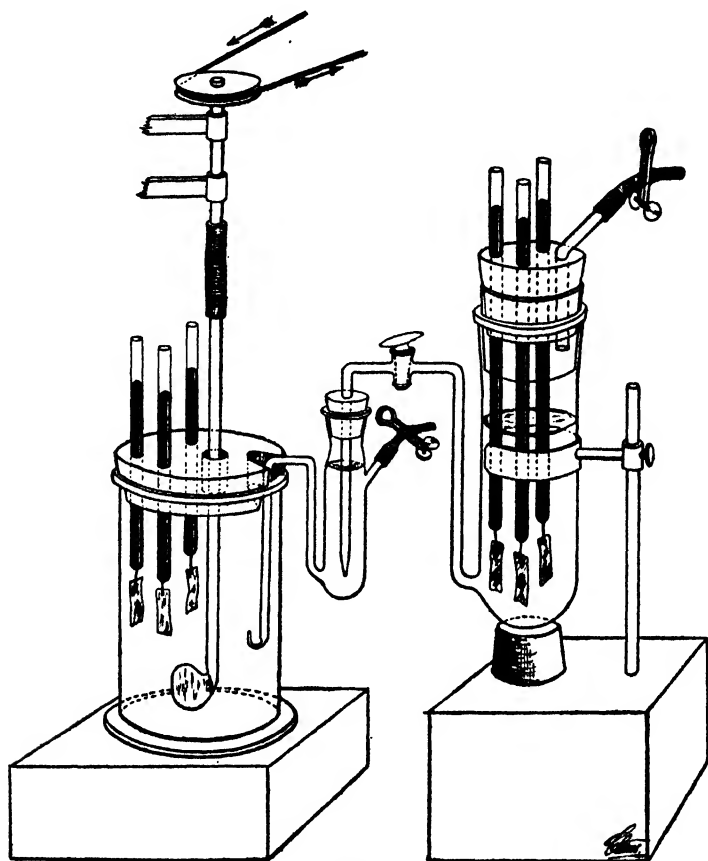

Fig. 2.

However, as the Höber-Hasselbalch platinated hydrogen electrode gave constant values when well shaken, the idea lay close at hand to try a strong stirring in the quinhydrone electrode to prevent the particles from sticking to the metal. The glass beaker in Fig. 1 was then substituted by a cylinder glass with an ordinary cork through which was inserted three smooth gold electrodes and in the centre a broad glass spatula, which was rotated rapidly. Further the cork had a fifth hole for the siphon-tube of the KCl-bridge (Fig. 2).

By this arrangement constant values were quickly obtained. The results are found in Table IV.

Table IV. *Measurements in buttermilk.*

	Quinhydrone electrode pH	Hydrogen electrode pH	Difference
(a)	4·68	4·66	+0·02
(b)	4·68	4·66	+0·02
(c)	4·66	4·70	−0·04
(d)	4·54	4·55	−0·01
(e)	4·61	4·57	+0·04
(f)	4·65	4·61	+0·04
(g)	4·78	4·76	+0·02
(h)	4·71	4·69	+0·02
(i)	4·71	4·69	+0·02
(j)	4·46	4·55	−0·09

Finally I shall mention some experiments on titrations of fresh and boiled full milk. My idea was to confirm the change in the reaction and buffer action by heating. A change in the reaction in alkaline direction —up to 0·20 pH units—has already been described by van Dam[4] and later by Milroy[5]. S. P. L. Sørensen and E. Jürgensen observed a change in pH by heating serum and egg protein solutions[6].

The titration has been made by adding lactic acid and the measurements were carried out simultaneously in two samples of the same full milk, the one fresh, the other heated ten minutes in a boiling water-bath and then left to cool to about 18°. The titration was made partly with constant volume, partly with increasing volume, in the following way:

(a) *Constant volume.* 80 c.c. milk + increasing quantities of lactic acid + water up to 100 c.c. is filled into glass beakers by means of pipettes. The reaction in each glass is measured as described, and the results are shown graphically in Fig. 3, where the pH values are marked along the abscissa and the number of millimol lactic acid per 100 c.c. fluid along the ordinate. For the most acid reactions it is necessary to use 5n lactic acid.

(b) *Increasing volume.* 100 c.c. milk is poured into a glass beaker, the reaction is measured, the glass beaker is taken away from the electrodes—and from a burette some cubic centimetres lactic acid is added; after stirring, the glass beaker is put back in its place and the potential read—the glass is removed, more acid is added etc. The volume is here increased up to 200 c.c.

With the quinhydrone electrode this titration is exceedingly simple and quick to make.

Table V. *Titration of full milk with lactic acid (constant volume).*

Millimol lactic acid per 100 c.c.	Fresh pH	Heated pH
0	6·65	6·63
2	5·63	5·64
4	4·84	4·84
10	3·71	3·71
20	3·20	3·20
50	2·62	2·64
100	2·29	2·32

Table VI. *Titration of 100 c.c. full milk with lactic acid (increasing volume).*

Millimol (=c.c. 1/n lactic acid)	Fresh pH	Heated pH
0	6·65	6·63
2	5·84	5·86
4	5·11	5·10
8	4·20	4·20
16	3·51	3·52
25	3·18	3·21
50	2·77	2·76
100	2·44	2·45
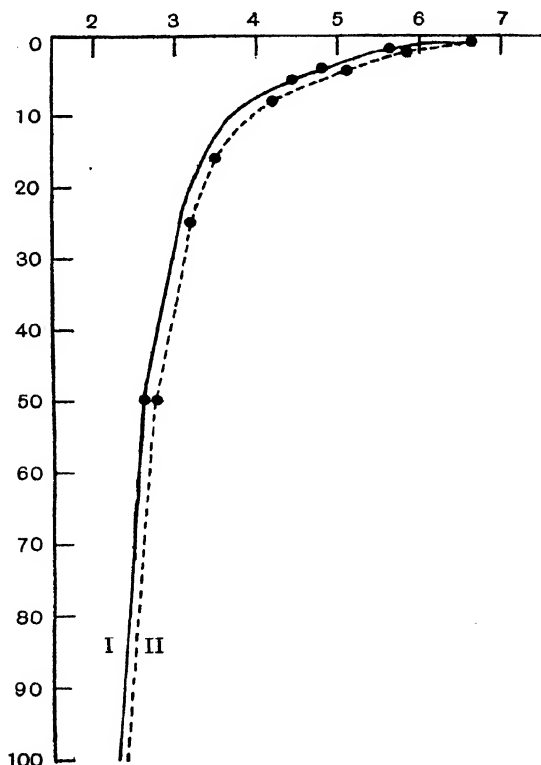
Fig. 3. Titration curves for fresh and heated milk.
I. Constant volume.   II. Increasing volume.

SUMMARY.

In the above I have shown the result of a series of measurements of the hydrion concentration in milk, cream, buttermilk and whey, partly employing Biilmann's Quinhydrone Electrode, partly Höber-Hasselbalch's Hydrogen Electrode.

The results of the tests agree.

· Measurements have also been made in fresh and heated milk.

By these measurements I found no difference in the reaction or buffer action of the fresh and heated milk.

The Höber-Hasselblach method is rather slow. This is unfortunate as the reaction of the dairy products (especially whey) is apt to change when kept standing, even for a short time. By this method one also runs the risk of too high pH values owing to loss of carbon dioxide and it is therefore far from being an ideal one.

Biilmann's Quinhydrone Electrode is quick working, easy and simple and you avoid the above-mentioned sources of error. It is therefore to be preferred for measurements in the fluids in question. It is especially well adapted for titrations, as it makes possible a large number of measurements in a short time.

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